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The floral morphology and ontogeny of some Chinese representatives of orchid subtribe Orchidinae

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The flower structure and development of ten species in six genera of the orchid subtribe Orchidinae are described and illustrated by scanning electron micrographs. Particular attention is given to the structure of the gynostemium, which for most species is interpreted from ontogenetic data. All the species studied here share a series of features, e.g. the sequence of tepal and anther initiation, the shape and position of the anther, the presence of auricles and basal bulges, the three-lobed condition of the median carpel apex and the lateral lobes of the median carpel embracing the basal ends of the thecae. However, the form and structure of the three carpel apices are most varied in the later development stages or in the adult flower. The genus *Hemipilia* shows a series of peculiar characters that are quite different from those of the other genera in Orchidinae. The peculiar structure and development of the viscidia in both *Amitostigma* and *Neottianthe* indicate that both of them are different from other genera in Orchidinae. The adult floral morphology shows that the genera *Galearis* and *Chusua* are not congeneric with *Orchis*. The separation of the lateral lobes of the rostellum in most genera studied here as well as in the *Brachycorythis* group from South Africa suggests that this is the ancestral state in the subtribe Orchidinae. In contrast, the conjoining of lateral lobes in *Dactylorhiza* and *Orchis* is suggested as a derived character.

ADDITIONAL KEY WORDS:—*Amitostigma* – *Chusua* – *Galearis* – gynostemium – *Hemipilia* – *Neottianthe*.

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INTRODUCTION

The subtribe Orchidinae (Orchideae, Orchidoideae, Orchidaceae) comprises 34 genera and 371 species distributed primarily in the northern hemisphere, ranging from Asia to Africa (Dressler, 1993). In China, where it occurs mainly in the southwest, it is represented by 9–10 genera and about 123 species (Lang, 1999; Luo, 1999). One monotypic genus *Aceratorchis*, which is endemic to China, was recognized by Chen & Tsi (1998) and subsequently allocated to the genus *Orchis* by Lang (1999). Three genera—*Amitostigma*, *Hemipilia* and *Neottianthe*—have most species occurring in China.

Kurzweil (1987) confirmed that the lateral appendages of the gynostemium of the Orchideae are composed of appendages of the fertile anther (filament excrescence) and basal bulges that differentiate from the floral cup representing vestiges of stamens a_1 and a_2. Generally the basal bulges develop into the arch-like structure that connects the lip to the gynostemium. In another paper (Kurzweil & Weber, 1991), the floral morphology of six genera of Orchidinae from southern Africa was investigated and the floral structure shown to be basically similar to that of the European representatives. However, some unusual lateral gynostemium appendages were found in both *Holothrix* and *Bartholina* where the filament excrescences are missing and the lateral gynostemium appendages correspond entirely to the basal bulges. These observations indicated that it is unjustified to distinguish the subtribe Orchidinae from Habenariinae by the features of the stigma.

Dressler (1981, 1993) subdivided the subtribe Orchidinae into four tentative alliances mainly based on vegetative morphology, in particular the form of the root-stem tuberoids. The study of Kurzweil & Weber (1991) suggested that the fourth alliance which includes *Holothrix* and *Bartholina* forms a very distinctive group from other Orchidinae while *Brachycorythis* (in the second alliance) and *Neobolusia* (in the third alliance) are very similar in flower structure. The monophyly of the subtribe Orchidinae (excluding *Holothrix*) and the subdivision based on the tuberoid form by Dressler (1993) were supported by the parsimony analysis of the ITS sequence (Pridgeon *et al.*, 1997; Bateman, Pridgeon & Chase, 1997). Furthermore, they suggested that *Orchis s.l.* is polyphyletic with three distinctive clades and that some genera which share palmate tubers, such as *Gymnadenia* and *Dactylorhiza*, are in fact closely related (Pridgeon *et al.*, 1997). Szlachetko (1995) proposed six subtribes in the tribe Orchideae (nearly equal to Dressler's subdivision), and grouped those six subtribes into two evolutionary lines.

In China, species of the subtribe Orchidinae were treated mainly in taxonomic and floristic accounts (Tang & Wang, 1951; Tang, Wang & Lang, 1980, 1982; Lang, 1980, 1984, 1985, 1990, 1994, 1998, 1999; Lang & Tsi, 1978; Lang, Xi & Hu, 1997; Chen & Tsi, 1998; Chen, Tsi & Luo, 1999). Chen (1982) suggested that the monotypic genus *Aceratorchis* may be the most primitive member of the tribe Orchideae because it possesses a nearly regular perianth. However, others considered this as a peloric variant of some other genus (Dressler, 1993). Mainly based on the features of the stigma, Lang (1980) mentioned some intermediate types in the tribe Orchideae. For example, he suggested that *Habenaria purpureo-punctata* might be an intermediate type between *Hemipilia* and *Habenaria*, while *Platanthera stenantha* and *P. bakeriana* (=*Habenaria bakeriana*) represent an intermediate type between *Platanthera* and *Habenaria*. Chen (1999) did not distinguish subtribe Orchidinae and Habenariinae in Chinese Orchideae, but has not given any explanations.

The present study analyses the flower morphology of various Chinese species of the subtribe Orchidinae. Particular attention is focused on the gynostemium which yields most of the characters that have been emphasized in taxonomy. The results of the ontogenetic observations are compared with those in earlier papers (Kurzweil, 1987; Kurzweil & Weber, 1991).

MATERIAL AND METHODS

The study includes 10 species in six genera. Material was preserved in 'Kew Cocktail' (H_2O: alcohol: formaldehyde: glycerine = 15,33: 16: 1: 1). The species and the sources of the specimens are listed in the Appendix.

Material for SEM investigation was prepared according to the technique described by Gerstberger & Leins (1978); after dissection, the specimens were chemically dehydrated in dimethoxymethane, and dried in a Polaron critical point drier using CO_2 as a carrier gas without passing an intermedium. Gold sputtered specimens were subsequently observed in a Hitachi S-800 scanning electron microscope at 25 KV.

ABBREVIATIONS

The following abbreviations are used in the figures. They essentially follow Kurzweil & Weber (1991).

an	fertile stamen or anther (the median stamen of the outer staminal whorl in position A_1; the narrow basal extensions of the thecae holding the caudicles are termed 'anther canals', and abbreviated as 'can')
au	auricles (appendages of the filament of the fertile anther)
b	basal bulges
br	floral bract
cau	caudicle
l	lip (=labellum)
lc	lateral carpel apices
mc	median carpel apex
mstl	stigmatic part of the median carpel apex visible in the mature flower
p_1, p_2	petals
pl	placenta
ro	rostellum (non-receptive portion of the median carpel apex)
ro_c	central rostellum lobe
ro_l	lateral rostellum lobe
s_1	median sepal (=dorsal sepal)
s_2, s_3	lateral sepals
sp	spur
stg	stigma
vi	viscidium

OBSERVATIONS

Hemipilia

The genus is a small and very distinctive group in the subtribe Orchidinae with 13 species distributed from north-western Himalayas to Taiwan (Luo, 1999). It clearly has its main distribution centre in the Henduan Mountains, where three taxa have been recognized (Luo Yi-bo & Chen Sing-chi, in prep.). All the species have a very prominent rostellum which is the best character differentiating *Hemipilia* from related genera.

Lindley (1835) compared *Hemipilia* with *Platanthera* and *Orchis chusua* when he first described this genus based on the only species, *H. cordifolia.* Schlechter (1919) suggested that *Hemipilia* be placed in a position near *Orchis* in tribe Orchideae based on the presence of two separate bursicles in all species, while noting that the deep concave rostellum indicates affinities with *Habenaria.* Dressler (1993) placed *Hemipilia* in one of four tentative alliances which shares spheroid tuberoids, including *Amitostigma*, *Neottianthe*, *Ophrys* and *Orchis*. Szlachetko (1995) included *Hemipilia* as well as *Platanthera* and *Pecteilis* in his subtribe Platantherinae.

The floral morphology of *Hemipilia flabellata* Bur. et Franch has been described in detail by Luo & Chen (1999), and other species of this genus are very similar to it. Only the gynostemium morphology of *H. calophylla* Par. & Rchb. f. (=*H. kwansiensis* T. Tang et T. F. Wang ex K. Y. Lang) and *H. flabellata* Bur. et Franch is reported here. The sessile anther is erect with two parallel thecae which taper towards their base, and thus form prominent anther canals (Figs 1O, 2A). The anther canals project forward with slightly incurved top ends, and are almost as long as the lateral lobes of the rostellum (Fig. 2B, C). The thecae are separate with an apical short connective process (Fig. 1O). The lateral gynostemium appendages can be distinguished as two parts by their sculptured surface in the adult flower (Figs 1N, 2A). Their basal parts are remarkable, extending forwards and forming two horn-like structures on the tops of the front margins (Figs 1P, 2A). The rostellum is quite large with three lobes. The two lateral lobes extend forwards while the central lobe conspicuously projects upwards (Fig. 1O). They are completely separate and quite far apart, and bear viscidia near the top ends (Figs 1P, 2D). The viscidia are thus positioned on the inner sides of the wall of the cavity rather than directly above the spur-mouth as in other species of Orchidinae. This feature of *Hemipilia* which can be found in Habenariinae (Kurzweil, 1987; Kurzweil & Weber, 1991, 1992) is quite different from that in other Orchidinae where the tops of the lateral lobes are conjoined or adjacent, and the viscidia are positioned directly above the spur-mouth. The central lobe is an erect, tongue-shaped structure, located in front of the anther and as high as or higher than the latter (Figs 1O, 2A). Another peculiar feature of *Hemipilia* is that the prominent extending basal part of the lateral gynostemium appendage combines with the lateral lobe of the rostellum forming a shallow channel in which the anther canal is positioned (Figs 1M–O, 2B). The pollinium has a curved long caudicle and a two-fold viscidium (Fig. 2G–I). The stigma is a pad-like structure and is located in the cavity below the rostellum. It is slightly elevated from the surrounding gynostemium and the receptive stigmatic surface is composed of finger-shaped papillae (*sensu* Dannenbaum, Wolter & Schill, 1989) (Figs 1P, 2F).

Only the gynostemium development of *H. calophylla* is described here. The differentiation of the floral primordia is the same as that of *Dactylorhiza maculata* which was described by Kurzweil (1987) (Fig. 1A–D). Initially, the anther is a flat and erect process (Fig. 1E). The thecae of the anther are originally separate and remain so in the adult flower. The top of the anther is undivided in all stages. The tapering basal ends of the thecae are slightly outcurved in middle stage (Fig. 1I, L). Later they extend forward forming clear anther canals.

In early stages, the auricles originate from the back of the anther and develop

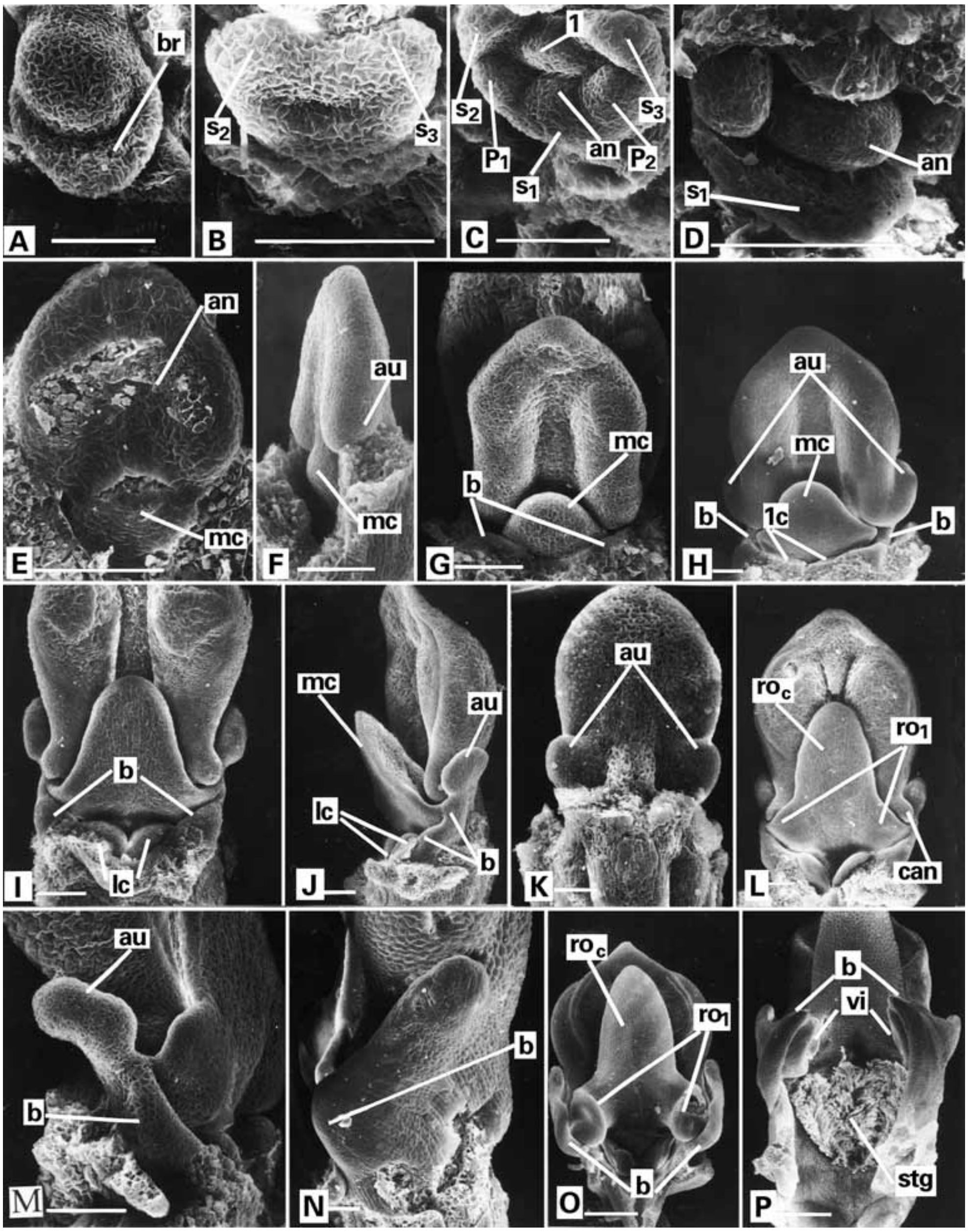

Figure 1. *Hemipilia calophylla*. (A–D) Initial differentiation of the floral primordium. Stages are shown in non-resupinate positions. Floral bracts are removed in (B–D). (E–P) Gynostemium development. Stages are shown in resupinate positions. (E) Anther in an early stage in ventral view. (F, G) Early development of the auricle and basal bulges. (H, I) Young gynostemium in ventral view, showing the three-lobed median carpel apex and the differentiation of the lateral carpel apices. (J) Side view of young gynostemium showing the fusion of the auricle, the basal bulge and the median stigma lobe. (K) Anther seen from the back. (L) Further development of the gynostemium. (M) Showing the lateral lobe of the median stigma lobe completely embracing the basal end of the theca. (N–P) Gynostemium in later stages. Scale bars. A–L = 0.1 mm; M–P = 0.5 mm.

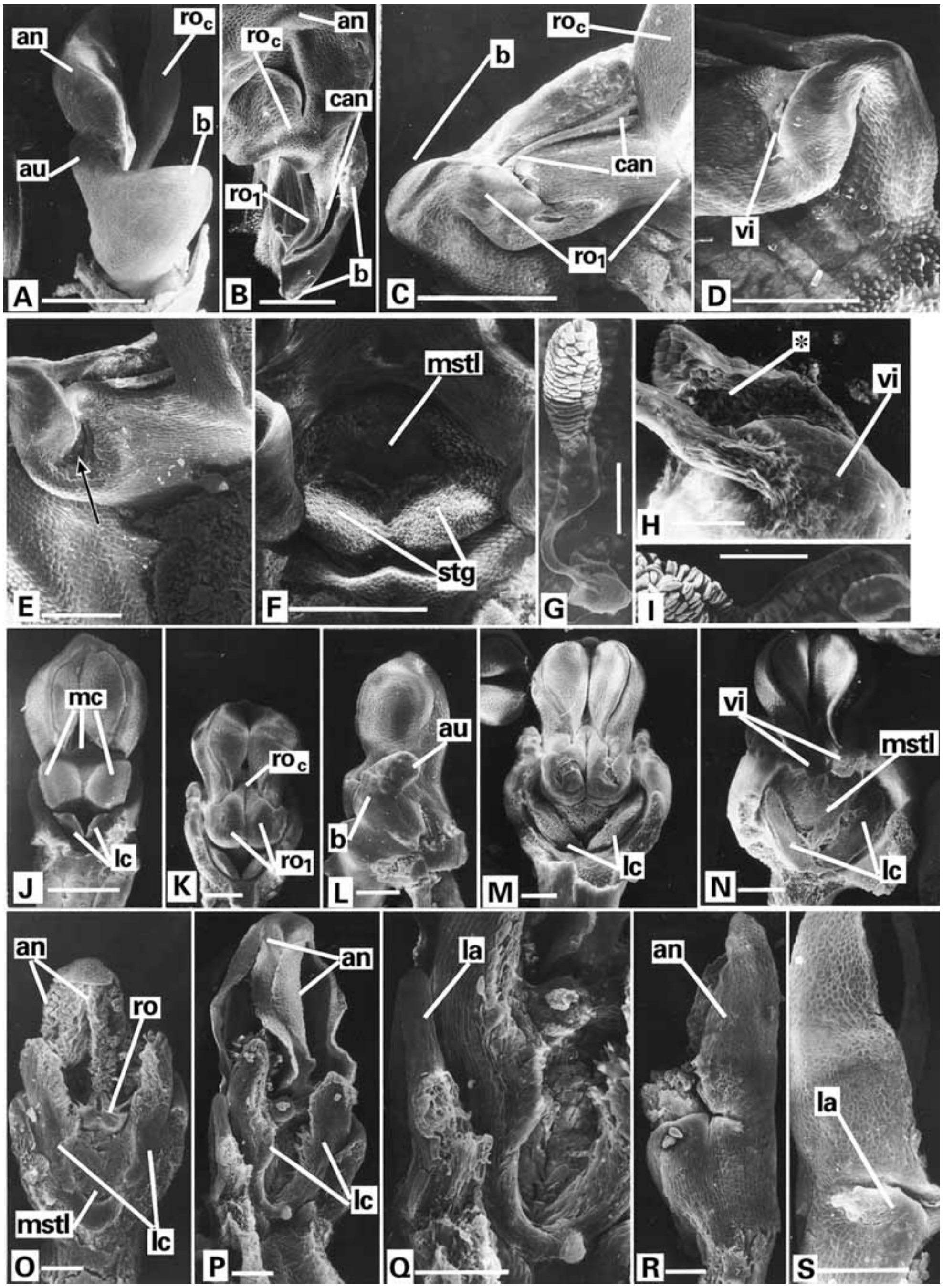

Figure 2. *Hemipilia flabellata.* (A–F) Different views of the gynostemium in later stage or in the adult flower. (G,H) Pollinium. The arrow in E shows the trace after the viscidium is removed. The star in H marks another side of the infolded viscidium. (J–N) *Orchis chusua*: gynostemium development. (O–S) *Orchis diantha*: gynostemium in the adult flower. Scale bars. A–C = 1 mm; H = 0.1 mm; I–S = 0.5 mm. All stages are shown in resupinate positions.

into large masses in the later development stages (Fig. 1F–M). At the same time, conspicuous basal bulges protrude from the receptacle in front of the anther (Fig. 1G–I). Subsequently, the basal bulges develop into two-lobed structures, and fuse with the auricles and the lateral lobes of the median carpel apex (Fig. 1I, J). In later development, they continue growing, and the upper parts completely fuse with the auricle while the lower parts extend forward with the anther canal and the lateral lobes of the rostellum (Fig. 1L–P). In the adult flower the auricles can only be distinguished by their sculptured surface (Figs. 1N, 2A). The basal bulges form two triangular teeth at the anterior upper margins, and are fused to the lip base on the front margin bases (Fig. 1P) and to the gynostemium on the posterior margins (Fig. 1N).

The primordium of the median carpel apex protrudes from the wall of the receptacle just below the anther in a very early development stage (Fig. 1E), and soon develops into a three-lobed mass (Fig. 1H). The central lobe, which is comparatively large in all stages, develops into an erect, tongue-shaped central rostellum in front of the thecae (Figs 1O, P, 2A). The lateral lobes are very small and extend into the space under the bases of the thecae in an early development stage (Fig. 1H); later, the margins are fused to the basal bulges and auricles (Fig. 1J). They then embrace the basal ends of the thecae (Fig. 1M) and project forward with the basal bulges, forming two shallow channels at the top position in which the anther canals are situated (Figs 1O, 2B).

The lateral carpel apices develop slightly later than the median one. They appear as completely separate structures until very late in their development (Figs 1O, 2F). The lateral carpel apices and a small portion of the median carpel situated directly below the rostellum join together to form a pad-like stigma in the adult flower (Fig. 2F). The stigma is slightly convex and covered with finger-shaped papillae (Fig. 1P).

Amitostigma

This genus is endemic to the mountain areas of East Asia. There is some debate about the number of species. In a taxonomic revision, Tang, Wang & Lang (1982) mentioned 20 species in this genus and recognized 19 species and one variety in China. Lang (1984) described one new species from Sichuan. Chen & Tsi (1998) noted 20 species in this genus, with 12 occurring in China, while Lang (1999) recognized 23 species, with 22 occurring in China. However, Dressler (1993) listed only ten species. The genus *Amitostigma* is closely related to the genus *Neottianthe*, but differs in having three free sepals (Lang, pers. comm.).

The floral morphology described here is based on *Amitostigma tetralobum* (Finet) Schltr. The flower is white or light pink in colour. The lateral sepals are spreading, while the dorsal sepal is erect and slightly converges with the petals forming a hood. The lip is deeply three-lobed and the central lobe has an emarginate apex. Its base extends backward into a spur that is as long as the ovary.

The sessile anther is erect with two parallel thecae which taper at their base but do not develop into canals (Fig. 3K). The thecae are very close to each other and lack a connective process. The lateral appendages of the gynostemium are relatively large and located next to the anther. Their upper portions are not pressed against the anther (Fig. 3M). The appendages can be recognized as composed of two parts by the quite different sculptured surfaces (Fig. 3J). The rostellum has an inconspicuous infolded central lobe and two rather large, separate lateral lobes bearing viscidia at

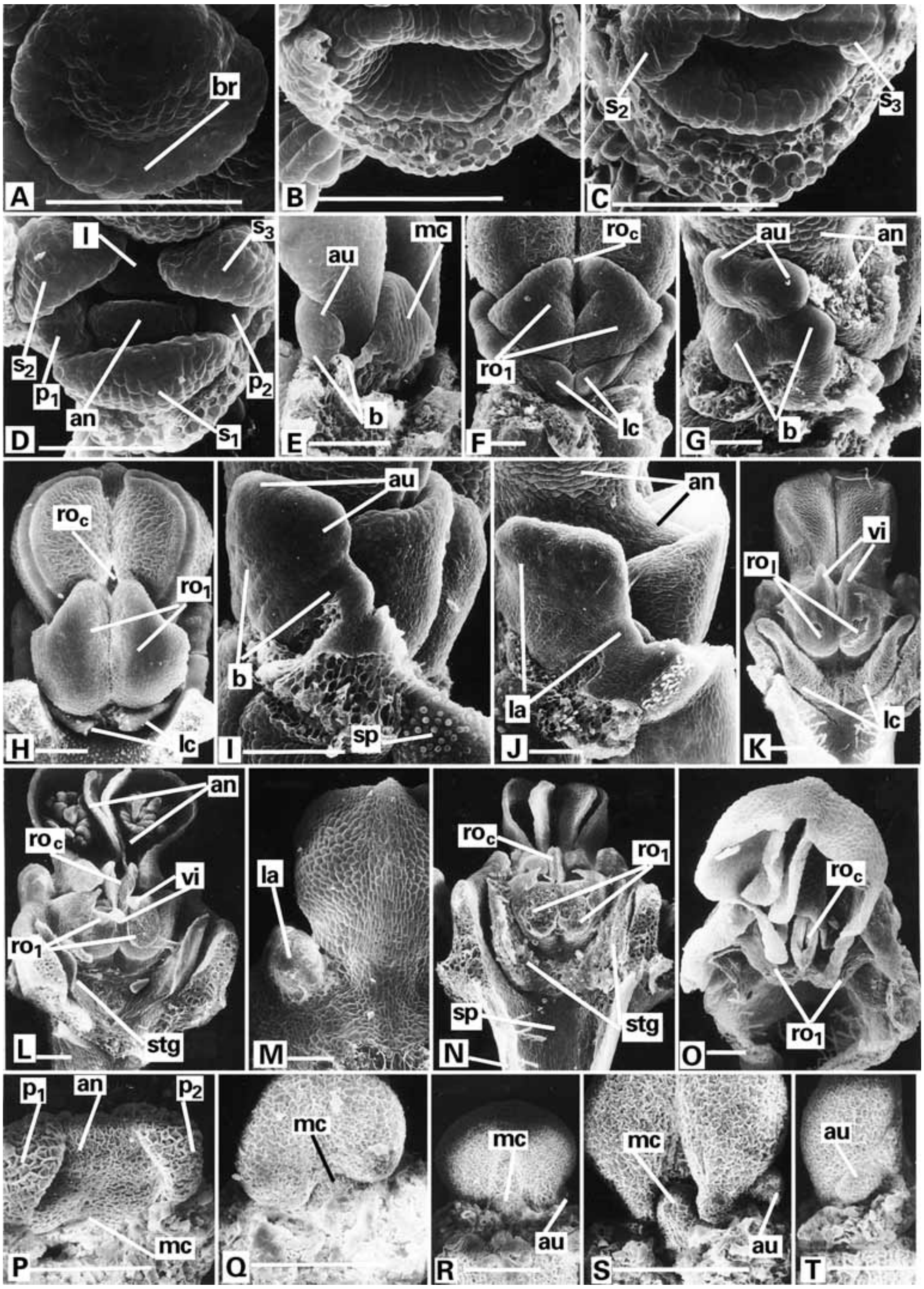

Figure 3. (A–O) *Amitostigma tetralobum*. (A–D) Initial differentiation of the floral primordium. Floral bracts are removed in (B–D). Stages are shown in non-resupinate positions. (E–O) Gynostemium development. (E,G, I, J) Young gynostemium in lateral view showing the development of the auricles and basal bulges. (F, H, K) Young gynostemium in ventral view showing the development of the three carpel apices. (L–O) Gynostemium in the adult flower. (P–T) *Amitostigma gracile*, young gynostemium in early stages. (E–T) Stages are shown in resupinate position. Scale bars. A–G = 0.1 mm; H–T = 0.2 mm.

the top margins near the base of the central lobe (Fig. 3L). The stigma consists of two very elongate pads derived from the lateral carpels, plus a small median stigma lobe. The elongate pads are fused at the base and form a 'U' in shape. The whole stigma is located in the cavity below the rostellum and is slightly raised from the surrounding gynostemium tissue (Fig. 3L, N).

Gynostemium development is described in *Amitostigma gracile* (Bl.) Schltr. and *A. tetralobum.* The features of organ inception on the floral primordium in *A. tetralobum* are similar to those in *Dactylorhiza maculata* (Kurzweil, 1987) (Fig. 3A–D). The auricles are obviously larger than basal bulges and are located at the sides of the gynostemium (Fig. 3E). The small basal bulges are directly positioned below the auricles except the anterior parts (Fig. 3E). The anterior petal margins are fused to the anterior outside margins of the basal bulges (which can be recognized by the broken tissue on the outsides of the basal bulges in Fig. 3E, G, I, J). Meanwhile the front bases of the basal bulges are fused with the lip (which also can be identified by the broken tissues). In middle development stages the auricles develop into a bilobed mass (Fig. 3G), and become a large mass without lobes in the adult flower (Fig. 3I). At the same time the whole basal bulge becomes a bilobed mass which is larger than the auricle and the posterior parts are larger than the anterior parts (Fig. 3G). In the late development stages it becomes a smooth mass without lobes and the posterior parts are completely fused with the auricle while the anterior parts are connected with the lip (Fig. 3I–K).

The median carpel apex appears as a three-lobed structure with a prominent central lobe in the early stage (Fig. 3E). The central lobe loses its prominent appearance and develops into a small fold of the infrathecal part (Fig. 3F, L, N). In contrast, the lateral parts become rather large and completely envelop the basal parts of the anther in the middle development stages (Fig. 3F). In later stages, they elongate slightly and form two pouches embracing the shortly tapering basal parts of thecae (Fig. 3J). Meanwhile, the lateral margins of the pouches are in contact with the lateral gynostemium appendages and are fused to it in the adult flower (Fig. 3J–L). The upper margins of the pouches grow into two small triangular structures near the base of the central lobe (Fig. 3K,L). This structure develops into the viscidia in the adult flowers. The lateral carpel apices are initiated as separate structures (Fig. 3F, G) and become fused to each other at the bases in later stages (Fig. 3H–L).

In *A. gracile* only one short ontogenetic phase was observed which is absent in *A. tetralobum.* The auricles originate from the back of the anther in early development (Fig. 3R–T). The median carpel apex is differentiated from the wall of the receptacle below the anther (Fig. 3P).

Neottianthe

In a comprehensive taxonomic revision, Lang, Xi & Hu (1997) recognized 12 species in this genus, all of them recorded in China. This genus is mainly distributed in the temperate and subtropical mountainous areas of Asia with one exception, *N. cucullata* (L.) Schltr. extending into north-eastern Europe. The flower structure of this genus differs from that of related genera in that the three sepals are erect and adnate forming a pointed cowl-like structure, but their tips are free. The lax spike with more or less one-sided flowers is also a typical feature of this genus.

Both floral morphology and gynostemium developmental features described here are based on only one species, *Neottianthe calcicola* (W. W. Smith) Schltr. The lanceolate sepals are adnate forming a pointed hood, and the linear petals are pressed on the dorsal sepal. The deeply three-lobed lip points forwards or slightly upwards. Its base is extended into a slender conical spur which is pointed downwards with a slightly swollen end.

In most respects, the gynostemium is similar to that of *Amitostigma tetralobum*. However, there are three different characters: (1) the auricles are obviously larger than the basal bulges at all stages (Fig. 4B–L); (2) the lateral lobes of the median carpel apex become oblong in shape, almost as long as the thecae, and their frontal faces develop into smooth viscidia in the adult flower (Fig. 4P); (3) only the outer surfaces of the lateral carpel apexes become receptive in adult flower (Fig. 4P).

The anther is erect in all stages. Initially, the auricles appear as oval-shaped bulges at the back of the anther (Fig. 4B–D). Later, they develop into two-lobed structures. Compared with the auricles, the basal bulges are relatively small at all stages. In the middle and late stages they develop into ridges which connect the gynostemium to the lip (Fig. 4L). The development mode of the median carpel apex is very similar to that of *Amitostigma tetralobum* in the early and middle stages (Fig. 4A–G). However, in the late developmental stages the lower parts of the lateral lobes of the median carpel apex extend downwards and cause the shape of the lateral lobes to change from triangular to oblong (Fig. 4J–O). In the adult flower the frontal faces of the lateral lobes become smooth, naked viscidia (Fig. 4P). The lateral carpel apices are initiated as separate structures (Fig. 4C–J), then elongate and fuse at the bases (Fig. 4M). Eventually they become receptive stigma in adult flower (Fig. 4P).

Gymnadenia

Gymnadenia, comprising about ten species, is of northern hemisphere distribution, and five species occur in China (Lang, 1999). The typical characteristics of this genus are: tubers palmately lobed with long and linear lobes; spur long and slender; gynostemium short with a long rostellum; viscidia two in number, long and linear without bursicle.

Due to the lack of fully mature flowers, only the gynostemium development of *Gymnadenia orchidis* Lindl. is shown here. Floral morphology is derived from the literature (Lang, 1987, 1999). Lateral sepals are spreading and angled downwards. The dorsal sepal is erect and incurved, forming a loose hood with the incurved petals. The lip has three lobes which are approximately the same in length and pointing downward with blunt tips. The spur is slender, curves downwards, and is longer than or equal to the ovary in length.

Gynostemium ontogeny (Fig. 5A–P) is similar to that of *Dactylorhiza maculata*. The only difference is the size of the lip primordium which is much smaller compared with other perigone primordia (Fig. 5D). However, this feature may be an abnormality.

Platanthera

This genus, with about 85 species, is the largest of north-temperate terrestrial orchids (Hapeman & Inoue, 1997). About 40 species occur in China, mainly distributed in the south-western mountainous areas (Lang, 1999). The Chinese members of this genus differ from other related genera in having a fresh, long and tongue- or strap-like lip, two separate or nearly parallel thecae with obvious

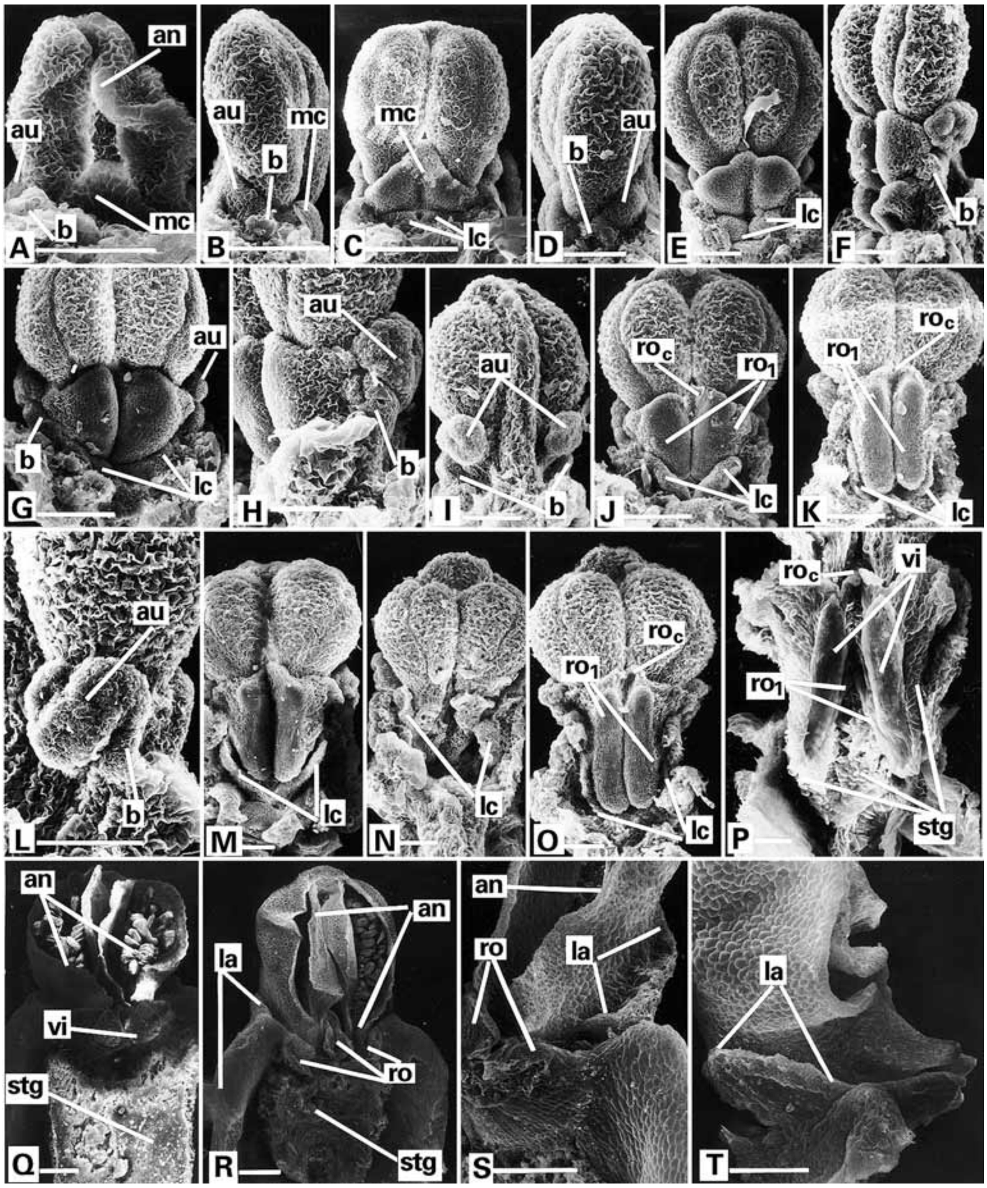

Figure 4. (A–P) *Neottianthae calcicola*. Development of the gynostemium. (A–E) Young gynostemium in early developmental stages. (F–J) Gynostemium in middle developmental stages. The basal ends of the thecae have been completely embraced by the lateral lobes of the median carpel apex as showing in F & H. (K–O) Gynostemium in later stages; the lateral lobes of the median carpel apex are beginning to be elongated in J & K. (P) Gynostemium in the adult flower. (Q–T) *Orchis brevicalcarata*; gynostemium in the adult flower. Scale bars. A–P = 0.1 mm; Q–T = 0.5 mm. All stages are shown in resupinate positions.

connective process, and most species lack stigmatic processes. In a newly described subgenus *Stigmatosea* K. Y. Lang, however, most species have two (rarely one) projecting stigmatic processes (Lang, 1998). This new subgenus includes 12 species

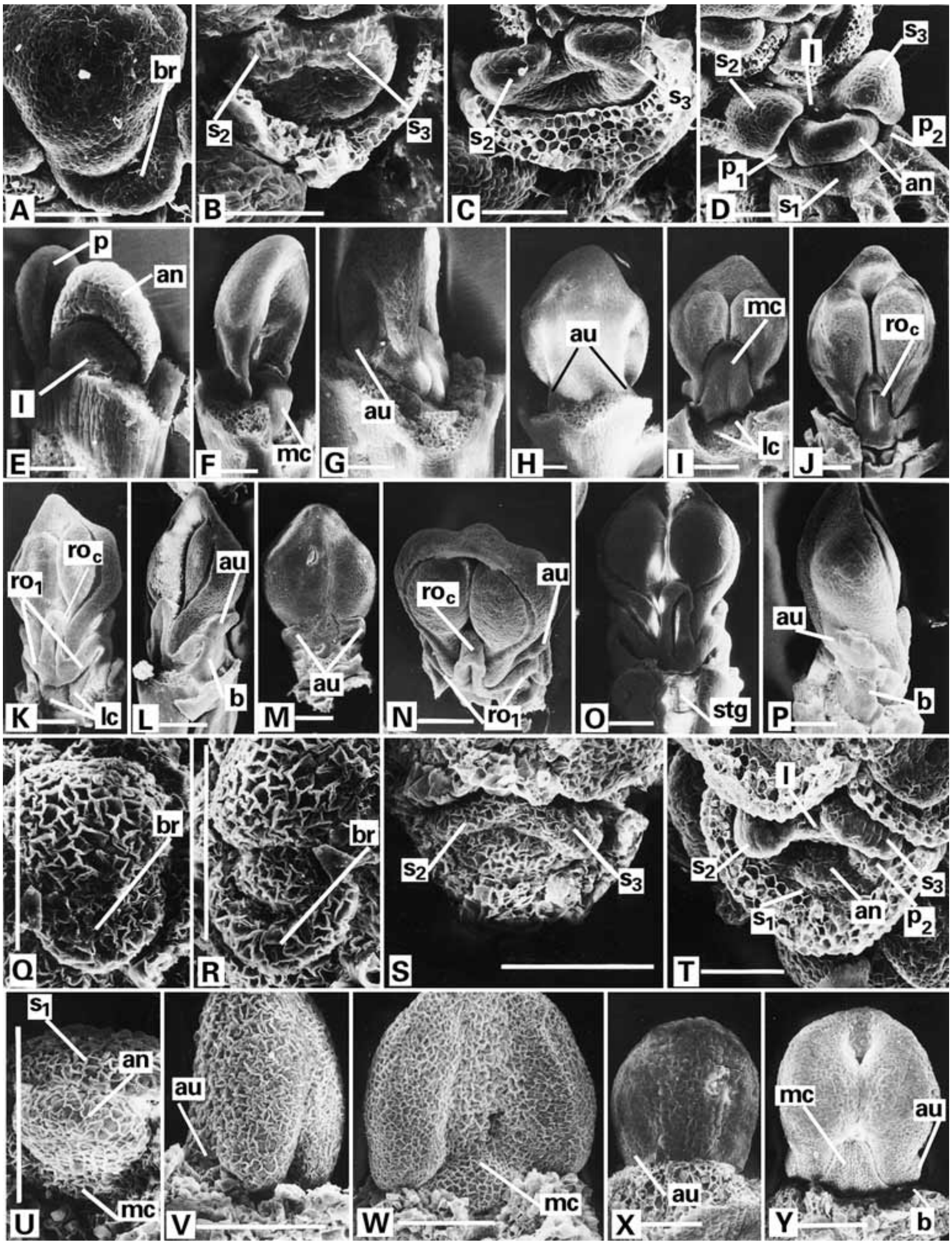

Figure 5. (A–P) *Gymnadenia orchidis*. (A–D) Initial differentiation of the floral primordium. Floral bracts are removed in (B–D). (E–P) Gynostemium development. (Q–Y) *Platanthera japonica*. (Q–T) Initial differentiation of the floral primordium. Floral bracts are removed in (R–T). (U–Y) Gynostemium development in early stage. Scale bars. A–H = 0.1 mm; I–J = 0.2 mm; K–P = 0.5 mm; Q–Y = 0.1 mm. (A–D & Q–T) stages are shown in non-resupinate positions; (E–P & U–Y) stages are shown in resupinate positions.

distributed in the Himalayas and Henduan regions. Hapeman & Inoue (1997) also noted that at least three species (*P. nivea*, *P. clavellata* and *P. integra*) from North America have stigmatic processes. Meanwhile, Hapeman & Inoue suggested that *Platanthera* is a monophyletic group with *Galearis* as the sister group based on the data from the nuclear ribosomal DNA internal transcribed spacers ITS1 and ITS2.

In the present study, only very young inflorescences of *Platanthera japonica* (Thunb.) Lindl. were available. Adult floral morphology is derived from the literature (Inoue, 1983). The dorsal sepal and petals incurve to form a helmet while the two lateral sepals are spreading and slightly angled backwards. The lip is narrowly deltoid and downward pointing, with a long, slender and deflexed spur at the base. The two thecae of the anther are parallel and nearly in contact with each other. Viscidia are narrowly deltoid, incurved and surrounded by the base of rostellum. The lateral walls of the gynostemium are well developed. The stigma is somewhat projecting.

The mode of differentiation of the floral primordia is similar to that of *Dactylorhiza macula* (Fig. 5Q–T). Gynostemium ontogeny in the early stages is largely similar to that of *Platanthera chlorantha* as described by Kurzweil (1987) (Fig. 5U–Y) except that the basal bulges appear on the receptacle and the two thecae are in contact with each other (Fig. 5Y).

Orchis

In taxonomic history, *Orchis* has been separated into six genera by some students (see Tang, Wang & Lang, 1980; Lang, 1987, 1999). These classifications were heavily reliant on tuber structures. Based on Chinese material, Tang, Wang & Lang (1980) and Lang (1987, 1999) emphasized that all those six genera shared a similar feature, in that they have a membrane outside the bursicles. The membrane can be easily ruptured at the slightest touch, upon which the viscidia become exposed. They even considered that the two bursicles in *Galearis* and *Chusua* noted by Nevski (1968) and Hunt (1971) may be the result of the rupture of the outside membrane. They therefore subdivided the genus *Orchis* in China into three sections rather than six genera.

According to Lang (1987, 1999), the genus *Orchis* comprises about 80 species, mainly distributed in the temperate regions of the northern hemisphere, extending throughout Europe to northern Africa, then across Asia to Japan and Taiwan in the east and the Himalayas in the south. There are about 28 species in China, most of them occurring in the south-western mountainous areas.

Due to limitation of suitable material, only the adult floral morphology of *O. chusua* D. Don, *O. diantha* Schltr. and *O. brevicalcarata* (Finet.) Schltr. is described here.

O. chusua. The dorsal sepal is erect and laterals spreading outward, while the petals curve inward to form a hood. The lip is three-lobed and pointing downward. The spur is longer than the ovary, cylindrical in shape and upward or horizontally pointing. Two thecae of the anther are closely parallel to each other but the basal parts are slightly separated (Fig. 2M, N). They taper toward the base where the viscidia are formed. The viscidia are located on the lateral rostellum lobes near the base of the central lobe (Fig. 2N). The lateral gynostemium appendages, as in *Dactylorhiza maculata* (L.) Soo, consist of the auricles and the basal bulges which can be identified by their different sculptured surface (Fig. 2L). The central rostellum lobe folds inwards and is obviously smaller than the lateral lobes (Fig. 2J, K). The lateral lobes of the rostellum are separate and their tops continuously project forward in late development, becoming viscidia in the adult flower (Fig. 2K, N). The stigma consists of three parts: two lateral carpel apices and one deltoid part derived from median carpel (Fig. 2M). It is situated in the cavity below the rostellum and slightly raised from the surrounding gynostemium tissue (Fig. 2N).

O. diantha. The three sepals are erect, separate and almost equal in size. Two petals slightly converge to form a loose hood. The lip is entire with a very short spur at the base. The anther is erect with two parallel thecae (Fig. 2O). The lateral gynostemium appendages are not clearly differentiated into two portions. However, from the slightly broken tissue (Fig. 2R) and the sculptured upper portion (Fig. 2S), it may be speculated that these appendages are the results of a high degree of fusion of auricles (upper portion) and basal bulges. The rostellum is small with an inconspicuous infolded central lobe and two slightly larger lateral lobes bearing the viscidia (Fig. 2J). The two lateral carpel apices strongly extend upward and at least one third is out of the cavity. They are fused at the bases to form a U-shaped structure and a small knob is present at the fused position (Fig. 2P, Q). The whole inner surfaces of the lateral carpels and the surface of the small receptive portion of the median carpel are covered by the long, finger-shaped papillae (Fig. 2Q). Both of them constitute the whole stigma.

O. brevicalcarata. The lateral sepals spread horizontally and are slightly angled at the lower margins. The dorsal sepal and petals are incurved to form a hood. The lip is slightly three-lobed and forward pointing. Its base is saccate and fused to the gynostemium. The anther is erect, and two thecae are parallel without a connective process between them and have canals at their bases. The lateral gynostemium appendages are clearly composed of auricles and basal bulges (Fig. 4T). The upper portions of the auricles are completely free from the anther (Fig. 4S). The basal bulges develop into the arch-like structures connecting the lip to the gynostemium. The rostellum is basically similar to that of *O. diantha* described above. However, the stigma is quite different and is a nearly square mass with smooth surface. It is located below the rostellum and raised slightly from the surrounding tissues.

DISCUSSION

Morphological conclusions

All species observed show some remarkably uniform characters in the early and middle development stages such as the sequence of tepal initiation, the form and position of the anther, the three-lobed condition of the median carpel apex and the presence of auricles and basal bulges. However, in later stages some peculiar features are developed; in particular, the form and structure of the three carpel apices vary extensively.

Perianth

Sepals with claws, lobes or spurs are not found in the Chinese species of Orchidinae. They are basically similar in shape, though somewhat varied in orientation, and are often separate except in the genus *Neottianthe* in which the three sepals cohere at their lower parts. Generally the galeate median sepal is coherent or adnate to petals to form a hood. Petals are always more or less erect without claws, lobes and spurs.

The lip varies greatly in shape, and usually has a spur or sac at the base but is not differentiated into a hypochile and an epichile, a feature which occurs in some South African species of *Brachycorythis*.

In the species we studied, the perianth, except for the lip, is usually not fused to the gynostemium at the base. In *Amitostigma tetralobum*, however, the anterior bases of the petals are fused to the gynostemium. By contrast, the petals are sometimes fused to the gynostemium in South African species of Orchidinae (Kurzweil & Weber, 1991). The lip is always fused on both margins to the gynostemium base. Generally the species we studied here show less variation compared with *Brachycorythis* and related genera in respect of the perianth.

Gynostemium

A gynostemium stalk is not found in any of the species studied here. The anther is erect and parallel to the axis of the gynostemium or the main part of ovary as suggested by Kurzweil & Weber (1991). The thecae of the anther are generally parallel and not separated by a prominent connective process. Elongate anther canals occur only in the genus *Hemipilia*. The gynostemium has obvious lateral appendages which were generally termed 'auricles' and interpreted as staminodes by many authors. However, according to their mode of development, Kurzweil (1987) and Kurzweil & Weber (1991, 1992) distinguished the auricles (filament excrescences) from the basal bulges (inner staminodes) in the lateral gynostemium appendages. This mode of development is further confirmed by our studies. Kurzweil (1987) suspected the existence of basal bulges in *Platanthera chlorantha*, and our study indicated that at least in *P. japonica* the basal bulges are present.

In all the species studied here, rostellum is obviously divided into a central lobe (infrathecal portion) and two lateral arms or lobes. Usually, the central lobe is a small erect rounded or triangular fold in most species, and is often more or less hidden by the lower thecal portions. However, in the genus *Hemipilia*, the central lobe becomes a very prominent, erect, tongue-shaped structure and it is even higher than the anther. Such a situation has not been found so far in other Chinese species of Orchidinae, but it has been found occurring in some South African species of Habenariinae (Kurzweil & Weber, 1992). In most cases the lateral lobes are parallel or adjacent and completely embrace the basal parts of the thecae, which is basically similar to that in the most southern African species of Orchidinae (Kurzweil & Weber, 1991). In *Hemipilia* they are completely separated and diverged which occurred only in some species of Habenariinae (Kurzweil, 1987; Kurzweil & Weber, 1992; Luo & Chen, in prep.). While in *Gymnadenia orchidis* they are fused together like those in *Dactylorhiza maculata* and *Orchis militaris* (Kurzweil, 1987). In the *Orchis* and *Gymnadenia* species examined here, the lateral lobes are fleshy and short bearing the viscidia at the tops (cf. *Dactylorhiza maculata*, *Orchis militaris* and the South African species of Orchidinae). However, in the genus *Hemipilia*, they project forward with the basal bulges, forming a shallow channel. The viscidia are located near the tops of the lateral lobes. Meanwhile, the lateral lobes of *Amitostigma* are slightly elongated and form two pouches embracing the basal part of the thecae. Their upper margins near the base of the central lobe grow into two small triangular structures where the viscidia are situated. In the genus *Neottianthe*, they become oblong-shaped and the whole frontal face develops into smooth, naked viscidia.

The stigma is situated in a cavity below the rostellum and is mainly derived from the lateral carpel apices, plus a portion of variable size of the median carpel. It is often referred to as entire, concave and is one of the critical features used to distinguish the subtribe Orchidinae from the Habenariinae (with convex, entire or two-lobed stigma and the lobes often stalked). The results of our observation indicated that in the species investigated here the really concave stigma does not occur. On the contrary, it is always a convex or pad-like structure more or less raised from the surrounding tissues. On the other hand, the shape of the stigma shows some variations in our study. In *Hemipilia*, *Gymnadenia* and *Orchis chusua* the stigma is a slightly convex or pad-like structure. In *Orchis diantha*, however, the stigma consists of two very elongate pads which are higher than the wall of the cavity. In both *Amitostigma* and *Neottianthe* a similar elongate pad-like stigma also occurs but it does not surpass the wall of the cavity. The similar elongate pad-like stigma was also reported in the genus *Schizochilus* from South Africa (Kurzweil & Weber, 1991). In *Orchis brevicalcarata*, the stigma is a smooth, nearly oblong, pad covered with a pellicle. In *Holothrix burchellii* and *H. secunda* from southern Africa a similar stigma appears (Kurzweil & Weber, 1991). The receptive surface is usually papillose in the Chinese species of Orchidinae, but smooth and covered with a pellice in *O. brevicalcarata.*

Phylogenetic considerations

There are always arguments about the extent and subdivision of the tribe Orchideae. Dressler (1993) divided the tribe Orchideae into two subtribes, Orchidinae and Habenariinae. The former has two or one basal viscidia which are attached to the interlocular caudicles and entire, concave stigma while the latter has the viscidia which are often borne on long rostellar stalks and entire or two-lobed, the lobes often stalked, convex stigma. Rasmussen (1985) provisionally subdivided the whole tribe Orchideae (Orchideae plus Diseae *sensu* Dressler, 1993) into three groups, and the main differences between the *Orchis* and *Habernaria* groups are the existence of bursicles at the base of thecae and the relative position of the thecae. However, according to the study of Kurzweil & Weber (1991), in the southern African genera of Orchidinae (*sensu* Dressler, 1981) the stigma is more or less convex rather than truly concave. As demonstrated above, in the species we studied the stigma is also not truly concave; in *Hemipilia* the viscidia are borne on long rostellar stalks, but the stigma is not convex. Meanwhile, Kurzweil & Weber (1991) reported that the lateral rostellum lobes in the southern African genera of Orchidinae are fleshy, very short and generally bearing the non-bursiculate viscidia. Based on our observations, we failed to trace any trails of bursicles in all species examined here, even in *Orchis chusua*, *O. brevicalcarata* and *O. diantha* despite the fact that Tang, Wang & Lang (1980), Lang (1987, 1999) believed that there are bursicles covering the viscidia in those three species. Szlachetko (1995) proposed six subtribes in Orchideae (*sensu* Dressler, 1993) mainly based on the features of the rostellum and anther. Furthermore, he grouped those six subtribes into two evolutionary lines: (1) Orchidinae, Herminiinae, Bartholiniae and Androcorytinae (initially adapted to bee-pollination); (2)

Platantherinae and Habernariinae (almost all the species pollinated by moth or butterfly). However, the result of the study by Luo & Chen (1999) suggested that *Hemipilia flabellata* is pollinated by bees rather than by moths or butterflies, and it utilizes a food deceit pollination strategy for pollination. Until now, this kind of pollination has not been reported in the genera *Platanthera* and *Habenaria*. Moreover, the structure of rostellum and the morphology of the anther in the late floral development stage are not similar, at least between *Platanthera chlorantha* (Kurzweil, 1987) and *Hemipilia calophylla*. Similarly, the evidence from the ITS sequence analysis has not supported the close relationship between *Platanthera* and *Habenaria* (Pridgeon *et al.*, 1997). The results of Pridgeon *et al.* (1997) showed that *Platanthera*, *Galearis* and *Pseudorchis* comprise a weakly supported clade. Also, based on the evidence of the ITS sequences analysis, Luo *et al.* (in prep.) suggested that *Hemipilia*, *Amitostigma* and *Neottianthe* comprise a strongly supported clade. Therefore, it seems likely that any subdivisions of the tribe Orchideae (*sensu* Dressler, 1993) based mainly on floral morphology are incorrect.

Based on floral morphology, Kurzweil & Weber (1991) recognized at least four groups in the southern African Orchidinae: (1) the *Brachycorythis* group, including *Schwartzkopffia* and *Neobolusia* (excluding *N. virginea*); (2) *Neobolusia virginea*, a species that does not resemble any other South African orchid, and was, therefore, separated as a monotypic genus *Dracomonticola* (Linder & Kurzweil, 1995); (3) *Schizochilus*, having no close relationship to other southern African Orchidinae; (4) the *Holothrix–Bartholina* group, a most distinctive group in the subtribe Orchidinae.

Dressler (1993) suggested the subtribe Orchidinae may be paraphyletic because both the Habenariinae and the Diseae may be a subclade of the Orchidinae. He recognized two basic alliances of taxa with tubers, one with elliptical to globose root tubers and another with palmate-attenuate root tubers. Dressler's suggestion has been supported by the evidence from the ITS sequence analysis based on 80 taxa of 17 genera in Orchidinae and seven taxa of four genera in Habenariinae (Pridgeon *et al.*, 1997). Meanwhile, Pridgeon *et al.* (1997) suggested that the Orchidinae are monophyletic excluding the genus *Holothrix*. *Dactylorhiza* (including *Coeloglossum*) and *Gymnadenia* (including *Nigritella*) are closely related, while *Galearis*, *Platanthera* and *Pseudorchis* comprise a weakly supported clade which may be related to the *Dactylorhiza–Gymnadenia* group. Furthermore, the genus *Orchis s.l.* is confirmed as a polyphyletic group and comprises three clades. Douzery *et al.* (1999) suggested that the genus *Brachycorythis* is closer to Habenariinae than to Orchidinae, and Pridgeon *et al.* (1997) believed this conclusion corresponds better to biogeographic patterns.

Although some data, especially the molecular ones, which are independent of the traits that have been the basis of previous classifications, have been published and some phylogenetic problems have been partly solved (Rossi *et al.*, 1994; Cozzolino *et al.*, 1998; Pridgeon *et al.*, 1997; Hapeman & Inoue, 1997), the whole phylogenetic problems of Orchideae are still far from clear. The following considerations on the relationship of the Chinese genera of the Orchidinae are mainly based upon the species dealt with here.

According to previous and present studies, among most genera of Orchidinae the two lateral rostellum lobes are conjoined or adjacent in adult flowers (Kurzweil, 1987; Kurzweil & Weber, 1991). Only in the genera *Hemipilia* studied here and *Platanthera* are they clearly separate (Kurzweil, 1987). From the view of floral development, the two lateral rostellum lobes originally occurred at two corners of the centre carpel apex in both Orchidinae and Habenariinae (Kurzweil, 1987;

Kurzweil & Weber, 1991, 1992; Luo & Chen, in prep.). They are obviously separated in early development stages, and become adjacent or conjoined in late stages. On the other hand, the adjacent or conjoined lateral rostellum lobes only appear in Orchidinae while separate lateral rostellum lobes occur in both Orchidinae and Habenariinae. Based on the present knowledge of the floral structures of Orchidinae and Habenariinae, we speculated that the conjoined lateral lobes of rostellum may represent a derived feature.

The results in the present study do not support the arguments of Tang, Wang & Lang (1980) and Lang (1987, 1999) that the genera *Galearis* and *Chusua* are congeneric with *Orchis s.l.*, and show that at least *O. diantha*, *O. chusua* and *O. brevicalcarata* should be separated. Except the feature of the lateral lobes of the rostellum, the stigma of *O. diantha* and *O. brevicalcarata* is also shown to be greatly different.

Some features of the genus *Hemipilia*, such as the elongated canals at the base of the thecae, the three lobes of the median carpel apex being heavily extended and the viscidia being borne on the long rostellar arms, are shared with *Habenaria* group. The feature of the basal bulges extending together with the lateral lobes of the median carpel apex to form two shallow channels is unique in the entire tribe Orchideae and apparently should be considered as a derived feature. However, the feature of the lateral lobes of the median carpel apex completely embracing the basal ends of the thecae is shared with other Orchidinae genera. Lang (1980) suggested that *Habenaria purpureo–punctata* may represent an intermediate type between *Hemipilia* and *Habenaria*. The detailed observation of the floral morphology of *Habenaria purpureo-punctata* (Luo & Chen, in prep.) showed that its gynostemium structure is obviously different from that of *Hemipilia*. Apparently, it is still difficult to assess the phylogenetic position of *Hemipilia* based on present floral morphological data.

Finally, both *Amitostigma* and *Neottianthe* are characterized by having a peculiar structure and development of the viscidia although whether *A. tetralobum* and *N. calcicola* can represent those two genera is still needed to confirm. Up until now, variation on the structure and development of viscidia has been observed only by Kurzweil (1990) in subtribe Disinae (*sensu* Dressler, 1981). Kurzweil (1990) recognized four types of viscidia formation among the Disinae, but none of them is similar to the viscidia we described here. So the peculiar viscidia in both genera must be considered as a derived character. In addition to the viscidia, the fusion of the anterior parts of the petals to the gynostemium in *Amitostigma* can be considered as a derived character in Chinese Orchidinae. On the other hand, the genus *Neottianthe* shows more derived characters than *Amitostigma* such as the peculiar stigma and the cohered sepals, which all are unique in the Chinese Orchidinae. Based on the present information, the closest relatives of *Amitostigma* and *Neottianthe* are still unclear.

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APPENDIX: LIST OF MATERIAL STUDIED

The following list cites all species examined in alphabetical order. Most species were identified by Professor Lang Kai-yun; *Hemipilia* species were identified by the authors. Voucher specimens of all species are deposited in the Herbarium, Institute of Botany, Chinese Academy of Sciences (PE), where all the material in the 'Kew Cocktail' is stored. Copies of voucher specimens of most species were also sent to the Herbarium, Royal Botanic Gardens, Kew.

Amitostigma gracile (Bl.) Schltr. – South-west Hunan Province: Luo Yi-bo s.n.

A. tetralobum (Finet) Schltr. – North-west Yunnan Province: Luo Yi-bo 25.

Gymnadenia orchidis Lindl. – South-east Xizang (Tibet) Autonomous Region: Luo Yi-bo 11.

Hemipilia flabellata Bur. et Franch. – North-west Yunnan Province: Luo Yi-bo 65.

H. calophylla (=*H. kwansiensis* T. Tang et T. F. Wang ex K. Y. Lang)—North Guangxi Zhuan Autonomous Region: Luo Yi-bo s.n.

Neottianthe calcicola (W. W. Smith) Schltr. – North-west Yunnan Province: Luo Yi-bo 9754.

Orchis brevicalcarata (Finet) Schltr. – North-west Yunnan Province: Luo Yi-bo 9734.

O. chusua D. Don – North-west Yunnan Province: Luo Yi-bo 79.

O. diantha Schltr. – North-west Yunnan Province: Luo Yi-bo 74.

Platanthera japonica (Thunb.) Lindl. – South-west Hunan Province: Luo Yi-bo s.n.