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Molecular phylogenetics and morphological reappraisal of the *Platanthera* clade (Orchidaceae: Orchidinae) prompts expansion of the generic limits of *Galearis* and *Platanthera*

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• *Background and Aims* The *Platanthera* clade dominates the North American orchid flora and is well represented in eastern Asia. It has also generated some classic studies of speciation in *Platanthera* sections *Platanthera* and *Limnorchis*. However, it has proved rich in taxonomic controversy and near-monotypic genera. The clade is reviewed via a new molecular phylogenetic analysis and those results are combined with brief reconsideration of morphology in the group, aiming to rationalize the species into a smaller number of larger monophyletic genera and sections.
• *Methods* Nuclear ribosomal internal transcribed spacer (ITS) sequences were obtained from 86 accessions of 35 named taxa, supplemented from GenBank with five accessions encompassing a further two named taxa.
• *Key Results* Using *Pseudorchis* as outgroup, and scoring indels, the data matrix generated 30 most-parsimonious trees that differed in the placement of two major groups plus two closely related species. Several other internal nodes also attracted only indifferent statistical support. Nonetheless, by combining implicit assessment of morphological divergence with explicit assessment of molecular divergence (when available), nine former genera can be rationalized into four revised genera by sinking the monotypic *Amerorchis*, together with *Aceratorchis* and *Chondradenia* (neither yet sequenced), into *Galearis*, and by amalgamating *Piperia*, *Diphylax* and the monotypic *Tsaiorchis* into the former *Platanthera* section *Platanthera*. After further species sampling, this section will require sub-division into at least three sections. The present nomenclatural adjustments prompt five new combinations.
• *Conclusions* Resolution of major groups should facilitate future species-level research on the *Platanthera* clade. Recent evidence suggests that ITS sequence divergence characterizes most species other than the *P. bifolia* group. The floral differences that distinguished *Piperia*, *Diphylax* and *Tsaiorchis* from *Platanthera*, and *Aceratorchis* and *Chondradenia* from *Galearis*, reflect various forms of heterochrony (notably paedomorphosis); this affected both the perianth and the gynostemium, and may have proved adaptive in montane habitats. Floral reduction was combined with lateral expansion of the root tubers in *Piperia* and *Diphylax* (including *Tsaiorchis*), whereas root tubers were minimized in the putative (but currently poorly supported) *Neolindleya–Galearis* clade. Allopolyploidy and/or autogamy strongly influenced speciation in *Platanthera* section *Limnorchis* and perhaps also *Neolindleya*. Reproductive biology remains an important driver of evolution in the clade, though plant–pollinator specificity and distinctness of the species boundaries have often been exaggerated.

Key words: *Aceratorchis*, *Amerorchis*, *Chondradenia*, *Diphylax*, *Galearis*, generic delimitation, internal transcribed spacer, *Neolindleya*, orchid, *Piperia*, phylogeny, *Platanthera*, *Pseudorchis*, speciation, *Tsaiorchis*.

INTRODUCTION

Platanthera Rich. (the butterfly orchids) is one of the most species-rich genera of North Temperate orchids (Hapeman and Inoue, 1997), having centres of diversity in East Asia (Inoue, 1983) and North America (Luer, 1975; Sheviak, 2002). *Platanthera* and allied genera share with many other temperate orchid groups a long and complex taxonomic history. Victorian luminaries including Robert Brown and Joseph Hooker lumped *Platanthera* species into *Habenaria*, misled by superficial floral similarities of these genera evident in, for example, rostellum morphology (cf. Dressler, 1993). Other

authors elected to divide *Platanthera* into several genera, each of which typically contained few (1–5) species. During the second half of the 20th century, the view most commonly expressed by morphological systematists recognized the majority of these former genera as sections of the genus *Platanthera* (e.g. *Lysiella*, *Limnorchis*, *Blephariglottis* and *Tulotis*). In contrast, other relevant taxa became more commonly recognized as distinct genera, notably *Pseudorchis* Seguier (syn. *Leucorchis* A. & D. Löve), *Piperia* Rydb. (1901*a*) and *Diphylax* Hook.f. (1869), together with the more obscure, monotypic *Tsaiorchis* Tang & Wang (1936). The most notorious 'taxonomic football' was *Piperia*, which was variously assigned to four other genera (Table 1b).

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Also relevant to this discussion are a small number of Eurasian species that were attributed by many authors to the dominantly European genus *Orchis* L., despite obvious floral and especially vegetative differences. Toward the end of the 20th century these species became more commonly assigned to the small, dominantly Eurasian genus *Galearis* Rafinesque (1833) and to two monotypic genera: the exclusively North American *Amerorchis* Hultén (1968) and the Chinese *Aceratorchis* Schlechter (1922). A similarly monotypic east Asian genus, *Neolindleya* Kraenzlin (1897–1904), was more commonly misassigned to *Gymnadenia*, again despite obvious floral and especially vegetative differences. Few if any authors suggested close relationships among (*a*) *Neolindleya*, (*b*) *Amerorchis* plus *Galearis*, (*c*) *Aceratorchis* or (*d*) *Pseudorchis* plus *Platanthera*; moreover, the supposed close relationship between *Pseudorchis* and *Platanthera* was frequently challenged, *Pseudorchis* often being attributed to *Gymnadenia* by authors who ignored several obvious floral differences (cf. Bateman *et al.*, 2006; Delforge, 2006).

Over the last decade, these intuitively inferred boundaries of, and relationships among, genera have been systematically revised (and, in many cases, overturned) by sequence-based phylogenetic analyses using the rapidly evolving nuclear ribosomal internal transcribed spacers (ITS) of nuclear ribosomal DNA (nrDNA). Pridgeon *et al.* (1997) and Bateman *et al.* (1997) identified a weakly supported *Platanthera* clade, consisting of *Pseudorchis* (one species analysed) as sister to *Galearis* (two species) plus *Platanthera* (six species, acting as placeholders for putative sections within this larger genus, estimated at >85 species by Hapeman and Inoue, 1997). These generic relationships were also obtained (again with limited statistical support) by Bateman *et al.* (2003), in a study benefiting from substantially increased taxon sampling. *Pseudorchis* (two species) was again shown as basally divergent, followed by the monotypic *Amerorchis*, which was placed as sister to *Galearis* (three species). The next branch showed the monotypic *Neolindleya* as sister to *Platanthera sensu stricto* (*s.s.*). Lastly, the genus *Platanthera* proved to encompass several former genera that were already incorporated within *Platanthera* by most authorities (represented by eight species). However, also nested within the genus was a strongly supported monophyletic group of the three sampled species of *Piperia*. Consequently, all ten *Piperia* species recognized by Ackerman and Morgan (2002) were duly incorporated in *Platanthera* as a new section of the genus (Table 4 of Bateman *et al.*, 2003).

In a parallel sequence-based project, Hapeman and Inoue (1997) produced an ITS tree for a better sampling (36 species) of *Platanthera s.s.*, though they omitted discussion of the identity and relationships of the outgroups. Their study recognized five sections within the genus. Four of these sections (*Tulotis*, *Lacera*, *Limnorchis* and *Blephariglottis*) were well supported as monophyletic. However, the fifth (and most species-rich) section, *Platanthera*, was very poorly supported, and none of the relationships inferred among the sections gained support from bootstrap values or decay indices.

More recently, DNA-based studies have sought to use genetic data to help explore speciation and hybridization in more narrowly defined groups of *Platanthera*, most notably the *P. dilatata* group (section *Limnorchis*) in North America (Wallace, 2003, 2004, 2006) and the *P. bifolia* group (section *Platanthera*) in Eurasia in general and England in particular (Bateman, 2005; Bateman *et al.*, 2009). The *P. bifolia* group is of particular significance because it generated one of the classic textbook examples of selection-mediated co-evolution between orchids and their pollinating insects (e.g. Nilsson, 1983, 1985; Maad and Nilsson, 2004; reviewed by Hapeman and Inoue, 1997; Bateman and Sexton, 2008), and has contributed to contrasting theories of instantaneous saltational speciation (summarized by Bateman and Rudall, 2006).

The present ITS analysis includes several additional species from *Platanthera* sections *Platanthera*, *Limnorchis* and *Lacera sensu* Hapeman and Inoue (1997), together with representatives of putative sections not recognized by Hapeman and Inoue (*Lysiella* and *Lysias*). It also includes two of the three commonly recognized species of the putative genus *Diphylax*, considered to be an upland endemic of the eastern Himalayas (Tang and Wang, 1940; Cribb, 2001). The purpose of the study is primarily to complete our revision of generic limits in the *Platanthera* clade, applying the principle of monophyly to the ITS data (where available) and reconsidering selected morphological characters across the clade. We also provide a better informed phylogenetic context for more focused ongoing evolutionary studies of the *P. dilatata* and *P. bifolia* aggregates. Lastly, we speculate briefly on speciation mechanisms, notably heterochrony, that appear to be operating within the *Platanthera* clade.

MATERIALS AND METHODS

Fieldwork and analytical materials

Data for the full ITS1–5·8S–ITS2 assembly were generated by the present authors from 86 accessions. Of these sequences, four were first published by Hapeman and Inoue (1997), nine by Pridgeon *et al.* (1997; also Bateman *et al.*, 1997) and eight by Bateman *et al.* (2003). We downloaded from GenBank a further four ITS sequences of *Platanthera* section *Limnorchis* selected from among a larger number deposited in 2006 by Lisa Wallace, and one sequence of section *Lacera* from the published study of Szalanski *et al.* (2001). A further three sequences were derived from the forthcoming doctoral thesis of R. K. Lauri. The remaining 62 accessions were sequenced specifically for the present paper and/or its companion (Bateman *et al.*, 2009). Most of the specimens were collected by R. Bateman, and the majority of the remainder by Y.-B. Luo (China) and Monicá Moura (Azores), though we also thank for provision of (typically single) samples M. Carine, M. Fischer, M. Hedrén, H. Lambert, R. Manuel, D. Nickrent, J. Tyler and J. Vogel.

Together, these 91 ITS sequences encompassed the following genera (*sensu* Bateman *et al.*, 2003): *Pseudorchis* (two accessions), *Neolindleya* (one), *Amerorchis* (one), *Galearis* (three), *Diphylax* (two), *Platanthera* section *Tulotis* (one), section *Lacera* (three), the controversial sections *Lysiella* (one) and *Lysias* (one), section *Limnorchis* (11 accessions of five species) and section *Piperia* (three). The previously well supported section *Blephariglottis* was not represented, nor

were three East Asian monotypic/near-monotypic genera: *Aceratorchis*, *Chondradenia* and *Tsaiorchis*. *Platanthera* section *Platanthera* yielded 50 sequences. These included single accessions of four molecularly distinct Oriental species. Of seven putative species of the Eurasian *Platanthera bifolia* group, two were represented by only one accession (*P. finetiana* and *P. metabifolia* from China) and one by just two accessions (*P. holmboei*: a reliable accession from Cyprus and a more ambiguous accession from Lesvos). The Azorean flora was represented by four accessions of *P. micrantha* and two accessions of the rarer *P. azorica*. In contrast, *P. bifolia* and *P. chlorantha* (the primary subjects of the companion study: Bateman *et al.*, 2009), together with their hybrids, were collectively represented by 42 accessions: three from China, nine from Continental Europe and the remaining 30 from Britain (Bateman *et al.*, 2009). As the *P. bifolia* group generated just seven closely similar ITS alleles, single exemplars only of each allele were carried forward to the parsimony analysis in order to simplify the tree-building procedure.

DNA extraction and sequencing

Total genomic DNA was extracted from silica-desiccated floral (or, less often, leaf) material from 75 specimens using the standard $2\times$ CTAB (cetyltrimethylammonium bromide) procedure (Doyle and Doyle, 1990) except that extractions were incubated in 500 mL of CTAB buffer, 50 mL of sarkosyl and 10 mL of proteinase-K. The rapidly mutating ITS region of nrDNA (e.g. Baldwin *et al.*, 1995; Hershkovitz *et al.*, 1999) was amplified via polymerase chain reaction using primers ITS4 and ITS5 (White *et al.*, 1990; Baldwin *et al.*, 1995) and cycling parameters from the earliest well-described phylogenetic analysis of subtribe Orchidinae, conducted by Pridgeon *et al.* (1997). Bidirectional sequencing was carried out on an ABI 3730 capillary DNA sequencer using an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer), employing the same primers used for amplification. Available resources did not permit cloning of ITS alleles.

Tree-building methods

Together, these 91 accessions generated 40 ITS alleles representing 37 named taxa. Exemplar samples for each allele are listed in the Appendix. Where particular species or infraspecific taxa yielded more than one allele, the exemplar samples are numbered; note that the alleles typified by the Homefield Wood specimen of *P. chlorantha* and the Morgan's Hill specimen of *P. bifolia* each represent large numbers of accessions of both species. In order to accelerate the tree-building procedure and to facilitate full rather than fast bootstrap analysis, trees were constructed only from these 40 alleles. Alignment was achieved by eye, yielding a total of 634 bp. All indels were coded as bistate characters. Each differentiable gap was coded separately, thereby maximizing the number of indels recognized (52) but also maximizing the proportion of those indels that functioned only as autapomorphies (32, = 62 %). Trees were constructed in PAUP 4·0b10 (Swofford, 2001) by heuristic search using

sub-tree pruning–regrafting (SPR) with MulTrees in effect, no limit on number of trees held and swapping on all trees, in order to recover all islands of most-parsimonious trees.

The primary analysis (A) identified *Pseudorchis* as the outgroup, following the topology of Bateman *et al.* (2003), and included the *Galearis* clade. Subsidiary experimental analyses omitted *Pseudorchis*, instead identifying the *Galearis* clade as outgroup (B), and omitted the *Galearis* clade (C). Each of the three analyses was conducted both with and without indels.

Robustness of nodes was explored via full bootstrap analyses using a full heuristic search with stepwise addition, permitting 1000 replicates. The large numbers of sub-optimal trees found in the primary analysis under collapse nodes with minimum length of zero, amb- (L = 30 trees, L + 1 = 969, L + 2 = approximately 10 000), limited calculation of decay index values to ≤3.

RESULTS

Of 692 characters, 325 were variable and 189 were potentially parsimony informative. By definition, all 52 indels were variable, but only 20 proved to be potentially parsimony informative. When indels were included, the primary analysis yielded a single island of 30 most-parsimonious trees, 671 steps in length with a consistency index of 0·55 (0·65 including autapomorphies) and a retention index of 0·80. Tree 19 is here reproduced as Fig. 1.

Support for all but two of the 31 internal branches could be categorized as either strong (bootstrap >95 %, decay index >3) or weak (bootstrap support ≤65 %, decay index ≤2). The weak nodes characterized either groups of closely related species that formed near-polytomies (*P. dilatata* group, *P. sparsiflora* group, section *Piperia* and *P. bifolia* group) or character conflict among major groups distributed along the spine of the cladogram. Four nodes collapsed in the strict consensus tree (arrowed in Fig. 1). Of these nodes, that within the *P. bifolia* group was relatively trivial, reflecting low levels of sequence divergence (Bateman *et al.*, 2009). However, the remaining three equivocal nodes were of greater potential importance. Possibly as a consequence of the absence of any representative of section *Blephariglottis*, section *Lacera* proved to be a serious source of instability in the central portion of the tree, occurring variously as sister to section *Limnorchis*, section *Tulotis* (*P. sonoharai*), or all sections other than *Lacera* and *Limnorchis* (Fig. 1). Section *Tulotis* was placed as sister to either section *Limnorchis* or all sections other than *Lacera* and *Limnorchis* (Fig. 1). Toward the base of the tree, *Neolindleya* could be placed as sister to either *Amerorchis* plus *Galearis* (Fig. 1) or the genus *Platanthera* (Efimov *et al.*, 2009).

Several tree-building experiments (topologies not shown) were conducted to explore the robustness of the trees resulting from the primary analysis. Reanalysing the primary matrix without indels generated a similar set of most-parsimonious trees, but caused *P. orbiculata* to diverge earlier in the topologies of the most-parsimonious trees, shifting from its previous position as sister to *P. oligantha* (section *Lysiella*) and relocating as sister to section *Lacera*. Omitting *Galearis* and *Neolindleya* reduced the number of most-parsimonious topologies to 18 and similarly shifted *P. orbiculata* down the

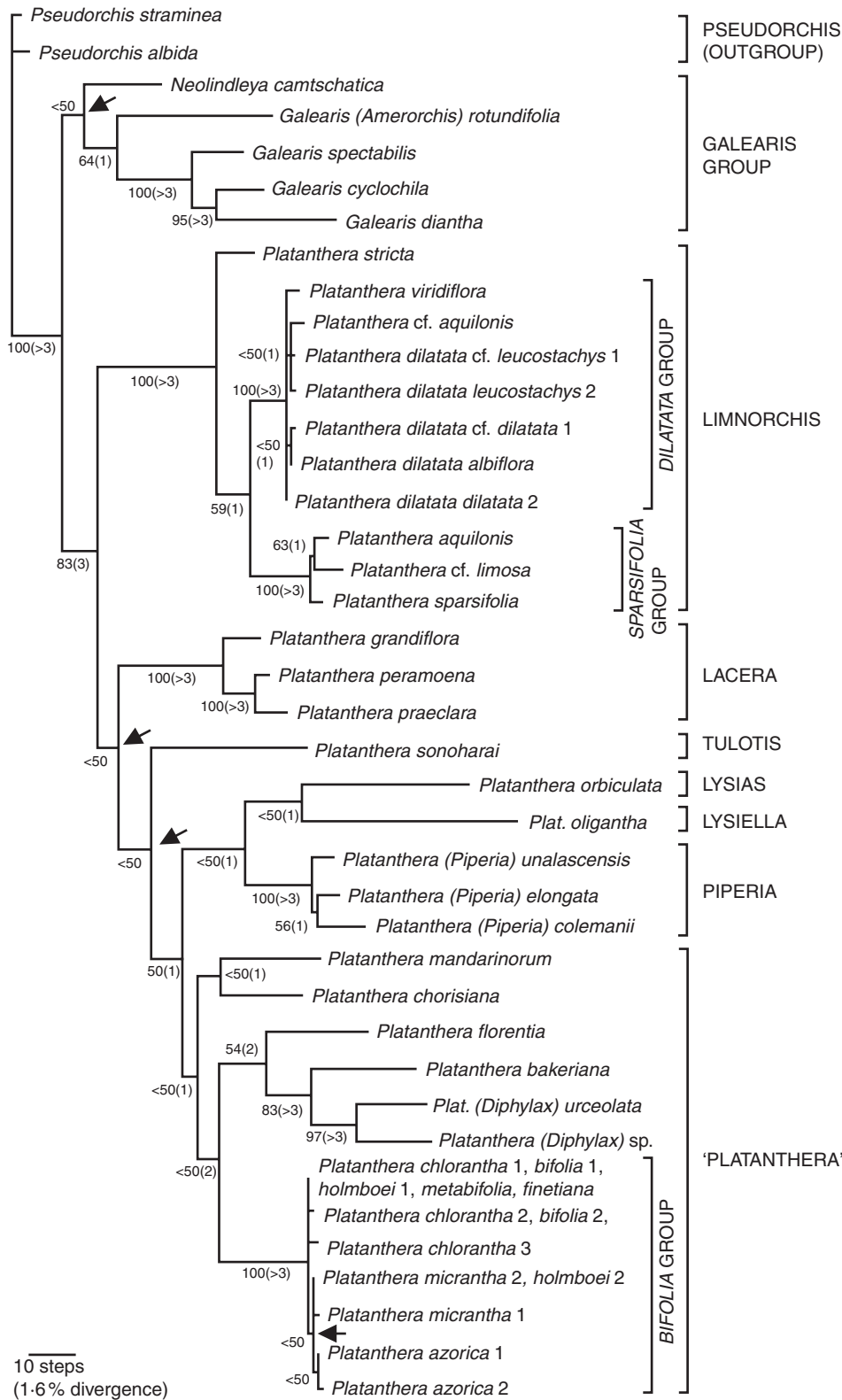*Bateman* et al. — *Phylogeny of the* Platanthera *clade*

FIG. 1. Preferred most-parsimonious tree of representatives of four of the five sections of *Platanthera* recognized by Hapeman and Inoue (1997: listed to the right), together with the former genera *Piperia* (Bateman *et al.*, 2003) and *Diphylax*, plus the two genera that constitute the revised *Galearis* group; the two *Pseudorchis* species constitute the outgroup. Branch lengths are proportional to Acctran optimization and include autapomorphies; figures on internal branches indicate bootstrap support and (in parentheses) decay index values. The four arrows indicate nodes that collapse in the strict consensus tree.

trees, to a location immediately above section *Lacera*. Omitting *Pseudorchis* and instead identifying the *Galearis–Neolindleya* clade as a multiple outgroup reduced the number of most-parsimonious topologies to 10. It not only placed *P. orbiculata* as sister to section *Lacera* but also placed this clade as sister to section *Limnorchis*, and identified section *Tulotis* as sister to this novel clade. In effect, the tree shown in Fig. 1 was re-rooted immediately above *P. sonoharai*. The main message of these experiments was that *P. orbiculata* is the greatest source of topological instability in the matrix.

DISCUSSION

The following discussion is based on, but not confined to, our preferred most-parsimonious tree based on our ITS sequences (Fig. 1). We regret that, after many years of searching, we have not yet acquired analysable DNA of the obscure (near-) monotypic Asian genera *Aceratorchis*, *Chondradenia* and *Tsaiorchis*. Consequently, our taxonomic treatment of these genera (Table 1) is based wholly on reconsideration of often-limited morphological evidence, and to some degree on the assumption that paedomorphosis of vegetative and/or floral organs is an iterative evolutionary theme within the *Platanthera* clade *sensu latissimo*. Further morphological and DNA-based studies are therefore desirable. Our conclusions regarding generic limits even deviate substantially from those established in *Genera Orchidacearum* (*GO*: Pridgeon *et al.*, 2001); we recommend amalgamation into other more widespread genera of three supposed genera that were recognized in *GO* (*Aceratorchis*, *Chondradenia* and *Diphylax*) and recognition of one genus that was omitted from *GO* (*Neolindleya*).

Delimitation and relationships of genera and sections in the Platanthera clade

The *Platanthera sensu lato* (*s.l.*) clade is undoubtedly sister to *Dactylorhiza s.l.* plus *Gymnadenia s.l.*, and is far more distantly related to *Habenaria*, despite the similar rostellar morphologies of the two genera (Bateman *et al.*, 2003). With the notable exception of *Pseudorchis*, mutation rate appears to be substantially higher in the *Platanthera* clade than in its sister group, the *Dactylorhiza s.l.–Gymnadenia s.l.* clade (fig. 1c of Box *et al.*, 2008). Within the *Platanthera* clade, it remains unclear whether *Pseudorchis* or the *Galearis* clade is sister to *Platanthera s.s.*, but it is clear that *Pseudorchis* merits the status of genus (*contra* Luer, 1975; Delforge, 2006). The two ITS branches that define the dichotomy between the *Galearis* clade (tentatively including *Neolindleya*) and *Platanthera s.s.* are only weakly supported, despite the fact that the two clades are readily separated using morphology.

Characters listed by Hapeman and Inoue (1997) as delimiting the genus *Platanthera* included its broad anther, undivided stigma and fusiform tubers, though reports of exceptions for each character have increased in number as the former genera *Piperia* and *Diphylax* have successively been incorporated into the genus (Bateman *et al.*, 2003; present study; also Lauri, 2007, who disagrees with this degree of 'lumping' at the genus level).

Within *Platanthera*, most of the sections recognized by Hapeman and Inoue (1997) survive the present analysis. However, the monophyly of section *Platanthera sensu* Hapeman and Inoue (1997) is challenged by the fact that the former genus *Diphylax* (and possibly also the former genus *Piperia*) is embedded deeply within it, and by the placement of *P. orbiculata* (attributed to section *Platanthera* by Hapeman and Inoue, 1997) as sister to *P. oligantha* of section *Lysiella* (Fig. 1). Admittedly, this placement is decidedly tentative. Several experiments with taxon and character sampling separated *P. orbiculata* from *P. oligantha* and placed it further down the tree. Also, *P. orbiculata* and *P. obtusata* (putatively a close relative of *P. oligantha*) were separated (albeit both occurring within section *Platanthera*) in the analyses of Hapeman and Inoue (1997). Lastly, *P. orbiculata* and *P. oligantha* are subtended by the longest branches in our tree, thus raising the possibility that their apparent sister-group status may reflect long-branch attraction.

Irrespective of their mutual relationships, valid cases can be made for recognizing the former genera *Lysiella* (here represented by *P. oligantha*) and *Lysias* (here represented by *P. orbiculata*) as sections of *Platanthera* (Fig. 1). The assignment of *P. orbiculata* to section *Platanthera* by Hapeman and Inoue (1997) is not supported.

The Pseudorchis clade

The ITS analysis of Bateman *et al.* (2003) revealed *Pseudorchis* to be a relatively short-branch taxon that caused topological instability within the *Dactylorhiza–Gymnadenia–Platanthera* clade. They noted its general morphological similarity to *Platanthera*, but also listed some morphological distinctions. These include spatulate leaves, the highly reduced central lobe to the labellum and a reduced gynostemium that minimizes the connective separating the two anther loculi.

Many authors consider the genus to be monotypic, whereas morphometric and allozyme analyses suggest that two closely related species may occur in Scandinavia (Reinhammar, 1995; Reinhammar and Hedrén, 1998). Although Bateman *et al.* (2003) considered the ITS divergence between the two putative species to be worryingly low (Fig. 1), it exceeds the level of divergence recorded here among members of the *Platanthera bifolia* aggregate (see below).

The Galearis clade: Galearis s.l.

The taxonomic challenges presented within the *Galearis* clade are analogous to those previously presented by the Eurasian *Himantoglossum* clade (Bateman *et al.*, 2003), in which a core group of several species of *Himantoglossum s.s.* had as its unambiguous sister the possibly monotypic, morphologically similar *Barlia*. This clade in turn had as its unambiguous sister the unequivocally monotypic, superficially morphologically distinct *Comperia*, and this clade in turn had as its ambiguous sister the unequivocally monotypic, more substantially morphologically distinct *Steveniella*. Each former genus was separated by a moderate degree of disparity in ITS sequences. Thus, Bateman *et al.* (2003) chose to sink both *Barlia* and *Comperia* into *Himantoglossum*, but retained *Steveniella* as a separate genus

because of its less confident phylogenetic placement and its comparatively distinct morphology (small, purple-washed leaves, central lobe of the labellum not further divided, spur apex clearly bifid, gynostemium compact).

In the case of the *Galearis* clade, *Amerorchis* mimics the role of *Comperia* and *Neolindleya* mimics the role of *Steveniella*. Both genera are commonly viewed as monotypic. *Amerorchis* is undoubted sister to *Galearis s.s.*, though they are separated by a substantial molecular distance. However, comparison of the morphological descriptions of the two genera given in recent publications (e.g. Wood and Neiland, 2001*a*, *b*; Sheviak and Catling, 2002) showed the two supposed genera to be similar in most characters. Moreover, characters that do show some difference are those that are known to be highly homoplastic within subtribe Orchidinae (Bateman *et al.*, 2003), notably *Amerorchis* more often having only one expanded leaf rather than two, and tending to have a more clearly three-lobed labellum. Gynostemium morphology differs between *Amerorchis*, North American *Galearis s.s.* (i.e. *G. spectabilis*, the type species of the genus) and southeast Asian *Galearis s.s.* (Luo and Chen, 2000; Perner and Luo, 2007; R. Lauri, unpubl. res.). *Amerorchis* possesses an undivided bilobed bursicle and *G. spectabilis* a divided bursicle, whereas Asian *Galearis* lack a bursicle (Perner and Luo, 2007). The stigma is divided into two zones separated by the rostellum in both *Amerorchis* (R. Lauri, unpubl. res.) and southeast Asian *Galearis s.s.* (Perner and Luo, 2007), whereas it delineates a single inverted triangle in North American *Galearis s.s*. In addition, *Amerorchis rotundifolia* has a distribution in North America that largely complements that of the more southerly *Galearis spectabilis*, exhibiting only a narrow zone of overlap. Their successive divergence below the two Asian species of *Galearis* in Fig. 1 implies that the Asian species are derived relative to the North American species (Bateman *et al.*, 2003).

Considered together, these factors arguably suggest that *Amerorchis* is better incorporated into *Galearis* than retained as a monotypic genus. The relevant combination is made here in Table 1; surprisingly, this species has not previously been placed in Rafinesque's (1833) *Galearis*, but rather was variously assigned to *Habenaria*, *Orchis* and (more credibly) *Platanthera*, before being isolated as *Amerorchis* as recently as 1968 (Hultén, 1968).

Luer (1975) and Sheviak and Catling (2002) recognized only one North American and one east Asian species of *Galearis* (excluding *Amerorchis*), whereas Wood and Neiland (2001*b*) suggested 3–6 species in total, Cribb and Gale (2009) listed five species (including the former genus *Aceratorchis*: see below) and Perner and Luo (2007) recognized six species in China alone. Expanding his geographical coverage, Hunt (1971) listed 11 species in Asia alone (including *Aorchis sensu* Vermeulen, 1972). Our analysis shows substantial molecular divergence between the two east Asian species analysed, *G. cyclochila* and *G. diantha*, both of which appear morphologically distinct from *G. wardii* (illustrated from China as '*Orchis*' *wardii* by Chen *et al.*, 1999, p. 306). We suspect that there are substantially more than two *bona fide* species in the genus *Galearis*.

In addition, after a decade of failing to obtain DNA samples of the rare Chinese species *Aceratorchis tschilensis* Schlechter (1922), we have nonetheless taken the radical step of incorporating *Aceratorchis* into *Galearis*, an action suggested tentatively by Cribb and Wood (2001) and more firmly by Cribb and Gale (2009). [Perner and Luo (2007, p. 135) went one step further in suggesting that '*A.*' *tschiliensis* is conspecific with *Galearis roborowskii*.] Our decision to place *Aceratorchis* within *Galearis* is based partly on similarities in vegetative and gynostemium characters and partly on the hypothesis that the near-radial symmetry of the perianth of *Aceratorchis* and its spurless condition reflect an origin from 'wild-type' *Galearis* flowers via a heterochronic developmental shift termed pseudopeloria (Rudall and Bateman, 2002; Bateman *et al.*, 2003; Bateman and Rudall, 2006; Mondragón-Palomino and Theissen, 2008, 2009). If so, they constitute atavistic reversals, rather than showing the fundamentally primitive morphology inferred by Chen (1982) and Chen and Tsi (1987).

This heterochronic hypothesis remains in need of explicit testing, ideally via evolutionary–developmental genetics (cf. Box *et al.*, 2008). Nonetheless, we have applied the same logic when re-assigning to *Galearis* the two southeast Asian species formerly attributed to the genus *Chondradenia* (cf. Maximowicz, 1886; Finet, 1898; Makino, 1902; Schlechter, 1919; Handel-Mazzetti, 1936; von Soó, 1966; Hunt, 1971; Makawa, 1971; Vermeulen, 1972; Brieger *et al.*, 1973; Wood, 2001; Perner and Luo, 2007). (Alternatively, assignment to *Platanthera* was suggested in the case of '*P.*' *doyonensis*: Lang *et al.*, 1999.) Nomenclaturally, these species have a chequered history, having undergone several previous generic transfers and received at least two *nomina nuda* (Table 1); also, morphological descriptions contrast in key features (e.g. compare Vermeulen, 1972; Wood, 2001). We still lack DNA samples and chromosome counts for either species. They closely resemble *Galearis* in vegetative features, having highly reduced tubers, only 1–2(–3) expanded leaves, and compact, few-flowered inflorescences characterized by large bracts. Flowers are small and unhooded. Perianth differentiation is poor, though the labellum is slightly larger than the other tepals and bears a small globose spur (also a swollen apical appendix in the case of the Japanese species). Resupination is unreliable, characterizing some but by no means all individuals. Their columns lack a bursicle (thus resembling the Asian rather than the North American species of *Galearis*), and the pair of pollinia are connected by a single dorsiventrally elongate viscidium (viscidial fusion has occurred in other species within subtribe Orchidinae, most notably *Anacamptis pyramidalis*: Darwin, 1877; Lind and Linderborg, 1989).

Despite this potential reproductive synapomorphy apparently uniting the two species, their respective geographical distributions are both highly restricted and strongly disjunct ('*Chondradenia*' *fauriei* occurs in Honshu Island of Japan and '*C.*' *doyonensis* in Yunnan, southern-most China: Wood, 2001), suggesting that these two rare species could have arisen independently. We speculate that these species originated from within *Galearis* and acquired similar paedomorphic morphologies that fitted them for montane specialization, a hypothesis readily tested via molecular phylogenetics. They may be analogous to the former genus *Aceratorchis* in showing floral paedomorphosis, though the effects have been less radical in '*Chondradenia*'.

Neolindleya

The east Asian genus *Neolindleya* Kraenzlin (1899) (a genus that, to our surprise, was not recognized in *Genera Orchidacearum 2*: Pridgeon *et al.*, 2001) is most commonly attributed to *Gymnadenia* by southeast Asian authors and to *Platanthera* by Russian authors, but was shown by Bateman *et al.* (2003) on ITS evidence to be a member of the *Pseudorchis–Galearis–Platanthera* clade. In their tree it was placed immediately above the *Galearis* plus *Amerorchis* clade as sister to *Platanthera*, albeit without bootstrap support. Here, it proved equally parsimonious to once again place *Neolindleya* as sister to *Platanthera* or as sister to *Galearis s.l.* (including *Amerorchis*: Fig. 1).

Given this molecular ambiguity, Efimov *et al.* (2009) re-examined the morphology of *Neolindleya* in an attempt to arbitrate between the two competing molecular phylogenetic placements. Morphology strongly supports placement of *Neolindleya* as sister to *Galearis–Amerorchis*, which would permit recognition of a *Neolindleya–Amerorchis–Galearis* clade, readily distinguished by the derived condition of extremely reduced tubers (a condition otherwise unknown in subtribe Orchidinae) and its failure to produce nectar, in contrast with *Pseudorchis* and *Platanthera* (e.g. Box *et al.*, 2008). *Neolindleya* shows greater morphological divergence from *Galearis s.s.* than does *Amerorchis*, including possessing larger numbers of expanded, crenulate-margined leaves and a labellum that is long and parallel-sided with a truncated central lobe (Bateman *et al.*, 2003). Efimov *et al.* (2009) noted that the combination of friable massulae and reduction to a rudimentary state of the caudicles and bursicle renders *Neolindleya* facultatively autogamous. They also highlighted a previously reported chromosome number of $2X = 36–38$ (Sokolovskaya, 1960), which contrasts with the figure of $2X = 42$ that characterizes the remainder of the *Platanthera* clade.

This morphological and ecological divergence, combined with its uncertain phylogenetic placement, suggests that *Neolindleya* is best retained as a genus separate from *Galearis s.l.* (Efimov *et al.*, 2009).

The Platanthera clade: section Limnorchis

In the analysis of Bateman *et al.* (2003), section *Limnorchis* was represented by only a single placeholder, *P.* (*hyperborea*) *viridiflora*. Here, the 11 sequences of *Platanthera* section *Limnorchis* generated by us or by Wallace (2002) (Appendix) show moderate divergence and constitute a well-supported monophyletic group. Indeed, the morphological distinction between section *Limnorchis* and section *Platanthera* was recently reinforced by Gamarra *et al.* (2008), who documented significantly different seed types in the two sections. Our 11 accessions supposedly represent five species, and the five accessions of *P. dilatata* (all subtly molecularly different) represent three varieties *sensu* Luer (1975) and Sheviak (2002). The samples are resolved into three molecularly distinct groups, based respectively on *P. stricta*, *P. dilatata* and *P. sparsiflora* (Fig. 1). Despite their similarity in morphology (noted by Luer, 1975), we originally hypothesized that the substantial divergence between the *dilatata* (originally referred to

hyperborea) and *sparsiflora* groups reflected contrasting geographical foci; the former characterizes northwestern North America, whereas the latter, which appeared relatively derived, is concentrated in the uplands of Mexico and southwestern North America. However, the subsequent inclusion in our analysis of two accessions of *P. aquilonis*, a species widespread in temperate North America (Sheviak, 1999), may challenge this interpretation. The accession assigned to *P. aquilonis* by L. Wallace was placed firmly in the southerly *sparsiflora* group, whereas the Alaskan accession tentatively assigned to *P. aquilonis* by R. Lauri was placed within the northerly *P. dilatata* group (Fig. 1). Further molecular and morphological study of this species group is therefore required.

The three accessions of the *sparsiflora* group analysed show moderate levels of both sequence divergence (Fig. 1) and morphological divergence (Sheviak, 2002). If correctly identified, the Sedona population of *P. limosa* found by us represents a small northward extension of the dominantly Mexican range given by Luer (1975). Morphologically, it is differentiated from the similarly but more widely distributed *P. sparsiflora* by its smaller flowers, narrower gynostemium and a labellum bearing a comparatively long spur and a small basal callus convergent with that commonly found in section *Tulotis* (Luer, 1975; Hapeman and Inoue, 1997; Sheviak, 2002). *Platanthera aquilonis* is facultatively autogamous (Wallace, 2006) and has a dorsiventrally compressed gynostemium; previous morphological comparisons with *P. hyperborea s.s.* are contradicted by the placement of Wallace's sample but encouraged by the placement of Lauri's sample. Wallace (2003) observed approximately equal separation of *P. sparsiflora*, *P. aquilonis*, *P. dilatata* and *P. stricta* in a population-level analysis that combined inter simple sequence repeats (ISSRs) and randomly amplified polymorphic DNAs (RAPDs).

Because of the exceptional taxonomic instability surrounding the delimitation of *P. hyperborea* and its putative allies (here represented by *P. viridiflora* and *P. dilatata s.l.*: cf. Luer, 1975; Wood and Neiland, 2001*c*; Sheviak, 2002), we have chosen to refer to the sister of the *sparsiflora* group as the *dilatata* group (Figs 1 and 2). *Bona fide* species of the
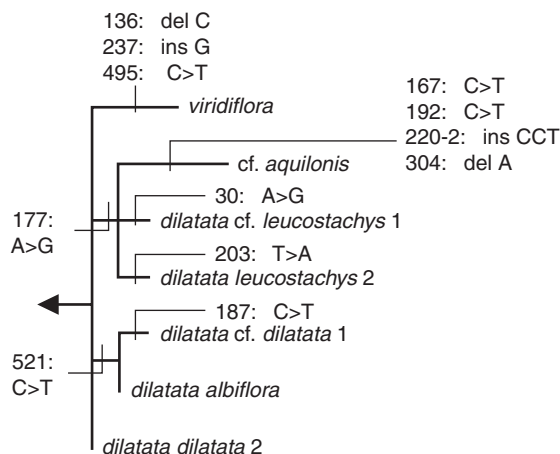

FIG. 2. Detail of topology for the *Platanthera dilatata* group, highlighting the position and nature of the small number of base pair changes that separate seven closely similar ITS alleles that differ by a maximum of eight character states (cf. Fig. 1).

dilatata group have distributions that stretch from the Aleutians in the north west southwards to California and eastwards through Canada and northeastern USA to Greenland, whereas *P. hyperborea* stretches to Iceland (Luer, 1975; Sheviak, 2002). Luer (1975) awarded *viridiflora* only varietal status under *P. hyperborea*, arguing that *viridiflora* was merely a more robust form of *P. hyperborea* with a longer labellum and a longer spur (greater than, as opposed to less than, 6 mm). However, Hapeman and Inoue (1997) revealed considerable ITS divergence between *P. hyperborea* and *P. viridiflora*, which did not prove to be sisters, thus strongly suggesting that *viridiflora* merits species-level distinction. It includes the western-most populations commonly attributed to the *P. hyperborea* group, occurring in Alaska, the Aleutians and Kamchatka (Luer, 1975; Sheviak, 2002). Supposed outlying populations of *P. viridiflora* in Hawaii have since been referred to *P. holochila*; they appear to be relatively distantly related to *P. viridiflora* within section *Limnorchis* (R. Lauri, unpubl. res.).

Certainly, the substantial divergence of *P. stricta* from the *P. dilatata* group challenges Sheviak's (2002) synonymization of *P. viridiflora* into *P. stricta*. Considerable molecular divergence between these species was also reported by Hapeman and Inoue (1997), though admittedly in our analysis it costs only one additional step to place *P. stricta* as sister to the *P. dilatata* group, despite the seven-step length of the branch separating *P. stricta* from the *P. dilatata* group (Fig. 1). The distribution of *P. stricta* coincides with those of *P. dilatata* and *P. hyperborea*, stretching from the Aleutians to northern California and Nevada. Morphologically similar to *P. dilatata* and *P. hyperborea*, it is best distinguished from them by its short, scrotiform spur (though the specimen analysed by us was the relatively slender-spurred '*gracilis*' morph).

Platanthera dilatata is shown as being only subtly molecularly distinct from *P. viridiflora* (Fig. 2), separated by a single base pair change plus two single base pair indels. The group was considered by both Luer (1975) and Sheviak (2002) to contain three varieties, differentiated by contrasts in the amount of green pigment in their flowers, overall flower size and the relative dimensions of the floral organs, notably the spur and the gynostemium. Sheviak (2002) speculated that these varieties show a significant degree of pollinator specialization. *Platanthera dilatata* var. *dilatata* is the most widespread taxon, stretching from Siberia across North America and southward to California and Utah, whereas var. *leucostachys* occurs only in the western half of the distribution of the nominate race, and var. *albiflora* has an even more restricted distribution across the Pacific northwest. The group was represented only by var. *leucostachys* in the analysis of Hapeman and Inoue (1997), but here it is represented by five accessions; of these, two (both of which were tentative identifications at the varietal level) were collected by us and three were downloaded from GenBank as randomly selected exemplars of 12 sequences deposited by Wallace (2002). No two of these accessions were separated by more than 4 bp differences (i.e. levels of divergence similar to that separating *P. viridiflora* from *P. dilatata s.l.*), but equally no two accessions were identical; all exhibited at least one sequence difference (Fig. 2). The modest phylogenetic structure revealed links

together the two samples of var. *leucostachys* but not the two samples of var. *dilatata s.s.*, one of which clusters with var. *albiflora* whereas the other appears to have the potentially ancestral ITS allele (Fig. 2). Both the molecular and morphological data suggest considerable complexity of relationships, consistent with the hybridization and allopolyploidization events recently reported in the group (e.g. Wallace, 2002, 2003, 2006). Understanding of this taxonomic section has improved greatly between the treatments of Luer (1975), Hapeman and Inoue (1997) and Sheviak (2002), a trend that will probably continue during the next decade (R. Lauri, unpubl. res.; L. Wallace, unpubl. res.).

Sections *Lacera* and *Tulotis*

Members of the distinctive section *Lacera*, recognized only relatively recently by Hapeman and Inoue (1997), have a fringed/toothed, three-lobed labellum and, in many cases, brightly coloured flowers (the first and third features are convergent with species in the well supported section *Blephariglottis*: Hapeman and Inoue, 1997). Our analysis included only three species of section *Lacera*. At this level of species sampling, the tree strongly supports *P. grandiflora* (northern Appalachians) as sister to *P. peramoena* (mid- to southern Appalachians) plus *P. praeclara* (US Midwest), a topology congruent with that obtained by Hapeman and Inoue (1997). Above section *Lacera* we have only the Oriental *P. sonoharai* as the placeholder for the widespread section *Tulotis* (cf. Hapeman and Inoue, 1997).

Sections *Lysiella* and *Lysias*

As discussed at the beginning of this section, we maintain reservations regarding the reliability of the sister relationships shown in Fig. 1 of section *Lysiella* and section *Lysias* to each other, and when combined as sister to section *Piperia*. Certainly, this clade would encompass an exceptionally broad range of morphologies, though it would permit the likely origin of section *Piperia* within North America. Support for both the clade consisting of sections *Lysiella*, *Lysias* and *Piperia* and the sister clade of section *Platanthera* is sufficiently weak that inclusion of any of the former sections in the latter cannot confidently be refuted.

Section *Piperia*

Piperia was previously widely recognized as a separate genus in North America (Luer, 1975; Wood and Neiland, 2001*c*; Ackerman and Morgan, 2002). However, although the ITS study of Bateman *et al.* (2003) clearly demonstrated that it was strongly supported as monophyletic, it showed equally convincingly that the clade was nested well within the genus *Platanthera* (see also Lauri, 2007). *Piperia* was therefore relegated to a section of the genus *Platanthera*.

The molecular branches subtending section *Piperia* and the clade that it forms with sections *Lysiella* and *Lysias* are no longer than those subtending other sections of *Platanthera* that were previously frequently recognized as separate genera (*Limnorchis*, *Lacera* and *Tulotis*). However, incorporation of

TABLE 1. *Taxonomic revisions of the former genera* Amerorchis, Aceratorchis, Chondradenia, Diphylax *and* Tsaiorchis, *plus a nomenclatural update on the former genus* Piperia

(a) Former genera *Amerorchis*, *Aceratorchis* and *Chondradenia*
Galearis rotundifolia (Banks ex Pursh) R.M.Bateman, **comb. nov.**
   Basionym: *Orchis rotundifolia* Banks ex Pursh, Fl. Amer. Sept., **2**: 588 (1814).
   Synonyms: *Habenaria rotundifolia* (Banks) Richardson, In Franklin: *Journey*: 750 (1823); *Platanthera rotundifolia* (Banks) Lindl., *Gen. Sp. Orchid.*: 292 (1835); *Amerorchis rotundifolia* Hultén, *Ark. Bot. ser. 2*, **7**: 34 (1968).
Galearis tschiliensis* (Schl.) P.J.Cribb, S.Gale & R.M.Bateman, **comb. nov.**
   Basionym: *Aceratorchis tschiliensis* Schl., *Rep. Spec. Nov. Regni Veg. Beih.* **12**: 329 (1922).
   Synonyms: *Orchis aceratorchis* von Soó, *Ann. Nat. Hist. Mus. Natl. Hung.* **26**: 350 (1929); *Orchis tschiliensis* (Schl.) von Soó, *Ann. Nat Hist. Mus. Natl. Hung.* **26**: 351 (1929).
*Galearis fauriei** (Finet) P.F.Hunt, Kew Bull. **26**: 172 (1971).
   Basionym: *Orchis fauriei* Finet, *J. de Bot.* **1808**: 340 (1898).
   Synonyms: *Chondradenia yatabei* Maxim., *Cat. Species Herb. Imp. Univ.*: 287 (1886), *nomen nudum* ex Makino, *Bot. Mag. (Tokyo)* **16**: 8 (1902); *Orchis yatabei* (Maxim.) Makino. *Ill. Fl. Jap.*: 704 (1948); *Chondradenia fauriei* (Finet) T.Sawada, *J. Jap. Bot.* **10**: 78 (1934), *nomen nudum*, ex F.Maekawa, Wild orch. *Jap. col.*: 456, 107 (1971).
*Galearis doyonensis** (Handel-Mazzetti) P.F.Hunt, *Kew Bull.* **26**: 171 (1971).
   Basionym: *Orchis doyonensis* Handel-Mazzetti, Symbolae Sinicae, *Anthophyta* 7: 1234 (1936).
   Synonyms: *Galeorchis doyonensis* (Handel-Mazzetti) von Soó, *Act. Bot. Acad. Sci. Hung.* **12**: 352 (1966); *Chondradenia doyonensis* (Handel-Mazzetti) Vermeulen, *Jber. Naturwiss. Ver. Wuppertal* **25**: 36 (1972); *Platanthera roseotincta* (W.W.Smith) T.Tang & F.T.Wang, *Bull. Fan Mem. Inst., Bot.* **10** (1940): 30 (see Lang *et al.*, 1999).

(b) Former genus *Piperia* (supplement to revision by Bateman in Bateman *et al.*, 2003)
Platanthera unalascensis (Spreng.) Kurtz, *Bot. Jahrb.* **19**: 408 (1894).
   Basionym: *Spiranthes unalascensis* Spreng., *Systema Vegetabilium* 3: 708 (1826).
   Synonyms: *Herminium unalasc(hk)ense* (Spreng.) Rchb.f., *Icon. Fl. Germ. Helv.* **13–14**: 107 (1838); *Platanthera foetida* Geyer ex Hook.f., *J. Bot. Kew Misc.* **7**: 376 (1855); *Habenaria unalasc(h)ensis* (Spreng.) S.Wats., *Proc. Amer. Acad. Arts* **12**: 277 (1877); *Piperia unalasc(h)ensis* (Spreng.) Rydberg, *Bull. Torrey Bot. Club* **28**: 270 (1901a).
Platanthera ephemerantha* R.M.Bateman, **nom. nov.**
   Synonyms: *Piperia candida* R.Morgan & Ackerman, *Lindleyana* **5**: 207 (1990); *Platanthera candida* (R.Morgan & Ackerman) R.M.Bateman, nom. illegit., *Bot. J. Linn. Soc.* **142**: 21 (2003) (illegitimate homonym of *Platanthera candida* Lindl., Gen. Spec. Orchid. Pl.: 295 (1835).
   Etymology: *Gr. ephemera* (transient) and *Gr. anthus* (flower): 'The flowers in *Piperia candida* are more completely white and more ephemeral than in any other member of the genus' (Ackerman, 2002, p. 574). The holotype of '*Piperia*' *candida* is retained, following Article 72.1a of the ICBN.

(c) Former genera *Diphylax* and *Tsaiorchis*
Platanthera urceolata (Hook.f.) R.M.Bateman, **comb. nov.**
   Basionym: *Diphylax urceolata* Hook.f., in Hook.f. *Icon.*, pl. xix (1889).
   Synonym: *Habenaria urceolata* (Hook.f.) C.B.Clarke, *J. Linn. Soc. Bot.* **25**: 73 + t30 (1889) ex Hook.f., *Fl. Brit. Ind.* 6: 165 (1896).
Platanthera uniformis?* T.Tang & F.T.Wang, *Bull. Fan Mem. Inst. Biol. Bot.*, **10**: 28 (1940).
   Synonym: *Diphylax uniformis* (T.Tang & F.T.Wang) T.Tang, F.T.Wang & K.Y.Lang, *Bot. Res., Inst. Bot. (Beijing)*, **4**: 11 (1989).
Platanthera contigua?* T.Tang & F.T.Wang, *Bull. Fan Mem. Inst. Biol. Bot.*, **10**: 31 (1940).
   Synonym: *Diphylax contigua* (T.Tang & F.T.Wang) T.Tang, F.T.Wang & K.Y.Lang, *Vasc. Pl. Hengduan Mount.*, **2**: 2526 (1994).
Platanthera neottianthoides* (T.Tang & F.T.Wang) R.M.Bateman & P.J.Cribb, **comb. nov.**
   Basionym: *Tsaiorchis neottianthoides* T.Tang & F.T.Wang, *Bull. Fan Mem. Inst. Biol. Bot.*, **7**: 131 (1936).

   * Species have not yet been subjected to molecular analysis.

the genus *Piperia* into the genus *Platanthera* has met greater resistance from orchid taxonomists, primarily because *Piperia* is delimited by morphological synapomorphies (notably short, inconspicuous caudicles and globose rather than fusiform tubers: cf. Luer, 1975; Ackermann, 1977, 2002; Wood and Neiland, 2001c; Ackerman and Morgan, 2002; Bateman *et al.*, 2003), whereas the genus *Platanthera*, as presently delimited, is not. However, we note that inconspicuous caudicles are a feature of several other genera in subtribe Orchidinae, and globose tubers are the plesiomorphic condition in the subtribe. Rather, it is the fusiform tubers of *Pseudorchis* and *Platanthera*, and their radical reduction in the *Galearis* clade, that constitute the most convincing synapomorphies (Box *et al.*, 2008; Efimov *et al.*, 2009). The globose tubers of section *Piperia* are ostensibly a reversion to the plesiomorphic condition within Orchidinae, though we suspect that careful ontogenetic studies would reveal developmental differences between the globose tubers in section *Piperia* vs. those of more distant genera such as *Orchis s.s.* We also note that field recognition (a critical element of any successful

classification) is dependent primarily on finding reliable differences between species in one or more character states; for identification purposes, it is unimportant whether these constitute synapomorphies.

Levels of sequence divergence between the three *Piperia* accessions included in Fig. 1 are consistent with their status as separate species. The history of species recognition in the section post-Rydberg (1901a, b) was summarized by Bateman *et al.* (2003, p. 21). However, the taxonomic revision given by Bateman in Table 4 of Bateman *et al.* (2003) requires further revision, as he failed to consider the possibility of accidentally creating orthographic synonyms when combining species and subspecies of '*Piperia*' into *Platanthera* (J. Zarucchi, pers. comm., 2003). These errors are here corrected in Table 1b. A current in-depth study by Lauri (2007) should provide a greatly improved understanding of the origin and evolution of section *Piperia*, as well as a much-needed test of previous hypotheses that the ten constituent species of the group originated relatively recently (Ackerman, 1977).

Section Platanthera

Section *Platanthera* was well sampled by Hapeman and Inoue (1997), who tentatively found the former sections *Lysiella* (epitomized by the circumboreal combination of *P. obtusata* and *P. oligantha*) and *Lysias* (epitomized by *P. orbiculata*) to be nested within section *Platanthera*. The consequently expanded section *Platanthera* was represented in their study by 15 species, but resolution among those species was sufficiently poor that, of the six groups that could be recognized, none had bootstrap support exceeding 50 % or a decay index >1. In addition, this study wholly lacked representatives of *Piperia* and *Diphylax*. Both former genera have since been shown to be nested within section *Platanthera* as broadly delimited by Hapeman and Inoue (1997) (Fig. 1), which can now be seen to constitute the largest and least resolved section within the genus.

The present analysis agrees with that of Hapeman and Inoue (1997) in suggesting that monophyly of section *Platanthera* is at best poorly supported, and that there is considerable molecular divergence among exclusively Asiatic species of the section (i.e. *P. mandarinorum*, *P. florentia* and *P. bakeriana*: cf. Inoue, 1983). The topology mirrors that of Bateman *et al.* (2003) in nesting (albeit with negligible bootstrap support) the Eurasian and European *Platanthera bifolia* aggregate within three Asian species of section *Platanthera* that are molecularly distinct but show only poorly resolved relationships. Moreover, the relationship of *P. mandarinorum* as sister to the group that extends from *P. florentia* to the *P. bifolia* group matches its placement in the topology of Hapeman and Inoue (1997), where *P. hachijoensis* and *P. ophrydioides* substitute for *P. mandarinorum* and *P. hookeri* (North American) substitutes for *P. florentia*.

Tentatively placed as sister to *P. mandarinorum* is *P. chorisiana*, which ranges from North Japan across the Aleutian islands to the Pacific Northwest of North America. This small boreal species produces a secund inflorescence of tiny, near-cleistogamous flowers that approach radial symmetry with a poorly developed labellar spur and gynostemium (e.g. Luer, 1975). It is therefore understandable that this species was assigned to *Limnorchis* by Anderson (1945), though this hypothesis is refuted by the ITS phylogeny, which is more consistent with the earlier decision of Nevski and Komarov in Komarov (1935) to separate this species as the novel genus, *Pseudodiphryllum*. Lastly, Fig. 1 places the Japanese *P. florentia* with moderate support as sister to the Sino-Himalayan *P. bakeriana* plus the Himalayan species of the former genus *Diphylax*.

The former genus Diphylax *and its relatives*

Diphylax was established as a genus by the younger Hooker (1889) but the taxon was soon downgraded by him to a section of *Habenaria* (Hooker, 1890). As summarized by Cribb (2001), *Diphylax* is a genus of three or four species and is confined to high elevations in the eastern Himalayas and southeastern China (Tang and Wang, 1940; Tang *et al.*, 1989, 1994). It resembles *Platanthera* closely in its tuber and vegetative characters, but its flowers are relatively small, compact and held in a secund inflorescence. The perianth approaches radial symmetry, showing only poor differentiation

in shape and size between the lanceolate sepals, lateral petals and labellum. The labellar spur is short and strikingly saccate. The column is bilaterally compressed, bearing distinct erect, cornute stigmatic lobes, elongate staminodes and a poorly developed rostellum. The pollinia have exceptionally short caudicles that contrast with the relatively large viscidium.

Cribb (2001) also advocated synonymization of the supposed monotypic genus *Tsaiorchis neottianthoides* Tang & Wang (1936) into *Diphylax*, convincingly arguing that the elongate, bifid, canaliculate rostellum of '*Tsaiorchis*' is a relatively minor modification of the condition previously described in *Diphylax* by Hooker (1890). These new nomenclatural combinations are here enacted in Table 1c.

The Platanthera bifolia *group*

The *P. bifolia* aggregate was represented by only a single accession of *P. bifolia* in the study of Hapeman and Inoue (1997) and by single accessions each of *P. bifolia* and *P. chlorantha* in Bateman *et al.* (1997), later supplemented with a sample of an eastern Mediterranean segregate of *P. chlorantha*, *P.* cf. *homboei* (Bateman *et al.*, 2003). Their study revealed only a single base pair separating *P. bifolia* from *P. chlorantha* plus *P. holmboei*, a level of divergence in ITS lower than that reported for any other putative species of *Platanthera* in any previous study.

The present study reports ITS data for 51 accessions of the *P. bifolia* aggregate, together representing seven putative species (including five of the six species of section *Platanthera* reputedly occurring in Europe plus Macaronesia: cf. Webb, 1980; Delforge, 2006). The aggregate is well supported as monophyletic in the ITS tree (Fig. 1). However, even given this greatly expanded sampling, levels of sequence divergence detected were exceptionally low, reaching a maximum in the 5 bp (0·7 % sequence divergence) that separated two Chinese accessions attributed to *P. chlorantha* from one of two sequences obtained from the Azorean endemic *P. azorica* (Bateman *et al.*, 2009). The only apparent phylogenetic structures detected within the *P. bifolia* group were two single-step branches: (1) a C > T transition at position 172 separated from the Azorean endemics *P. micrantha* and *P. azorica*, plus the sequence from the accession of *P. holmboei* from Cyprus, and (2) another C > T transition at position 629 that separated both accessions of *P. azorica* from all four accessions of *P. micrantha* (Fig. 1). Both branches attracted predictably low statistical support.

Overall, our 51 accessions of seven putative species yielded just seven ITS alleles. Three of these seven alleles (including the two most common) were found in more than one putative species, and the apparently plesiomorphic allele was found in no less than five of the seven putative species. Moreover, several individual accessions of *P. bifolia* and *P. chlorantha* were each observed to maintain two alleles (Bateman *et al.*, 2009).

Morphologically, the *P. bifolia* group shows levels of variation similar to those of most other sectional and subsectional groups within the genus *Platanthera*, at least in features such as overall flower size, labellum dimensions and pollinium presentation (Bateman *et al.*, 2009). One notable feature of the group is the unusually strong differentiation evident between the one or more often two expanded basal leaves and the bract-

like leaves that develop closer to the inflorescence. This feature is less noticeable in the Azorean species *P. micrantha s.l.* and *P. azorica* (e.g. Delforge, 2006), which have small, compact, green, relatively short-spurred flowers that are superficially more reminiscent of section *Limnorchis* than of section *Platanthera*. However, our data tentatively suggest that the species diverged recently, from within the *P. bifolia* group. Geographical proximity suggests that the most likely ancestor is *P. algeriensis*, a putative derivative of *P. chlorantha* endemic to northwestern Saharan Africa that we have not yet analysed molecularly.

Clearly, there is a strong discrepancy between the relative degrees of morphological and molecular divergence observed among species of the *P. bifolia* group. There are three possible explanations for this discrepancy: (1) they are not (yet) *bona fide* species; (2) they are *bona fide* species but evolved very recently; or (3) they are older species that have existed for a significant period of time but are still given collective molecular cohesion by ongoing gene flow through hybridization. These critical issues are explored at greater depth by Bateman *et al.* (2009).

Evolutionary trends in the Platanthera clade

In their benchmark study of the genus, Hapeman and Inoue (1997) mapped across their fairly well sampled tree of *Platanthera* species flower colour, dominant pollinator type and typical placement of pollinia on those pollinating insects, reporting substantial homoplasy in each of those character suites. Understandably, they emphasized floral convergence toward specific pollinators as the predominant speciation mechanism within the genus, noting that it 'has apparently undergone a tremendous radiation [that] represents nearly all of the non-deceptive pollination syndromes found in the Orchidaceae' (p. 436).

However, setting aside the spectacular colours and fringed labella of some North American species of sections *Blephariglottis* and *Lacera* (which are strongly positively correlated with preferred pollinators that possess colour vision), it is perhaps more remarkable how the genus has achieved those shifts of dominant pollinator using such a modest repertoire of floral changes. In the great majority of species, flower colour varies from green to 'white' (strictly, translucent), presumably depending on the concentration of chlorophyll in the flowers. Flower size varies considerably across the genus. Within the flower, the relative dimensions (and occasionally the postures) of the perianth segments and the shape of the gynostemium differ somewhat, but these are simple allometric shifts (Bateman and Sexton, 2008) that could readily be mapped and interpreted on a Thompson grid (Bateman *et al.*, 2009). Thus, perhaps the most striking feature of the taxonomic diversification in the genus is that it required such a limited repertoire of morphological innovations.

We agree with Hapeman and Inoue (1997) that pre-adaptation has most probably played a significant role in the evolution of *Platanthera*, but we also infer a major role for heterochrony, underpinning the relatively simple changes of relative shape and sizes of the organs within the flowers (Rudall and Bateman, 2002, 2004; Bateman and Rudall, 2006; Bateman *et al.*, 2009). Paedomorphic reduction of the perianth is evident in the former genera *Aceratorchis*, *Chondradenia*, *Piperia* and *Diphylax*, in which it is often accompanied by reduction in the relative size and complexity of the gynostemium. Overall, there are obvious similarities in the independent origins of broadly similar morphologies that led to the establishment of these four former genera, all apparently originating in montane areas (above 3000 m in the case of the three Southeast Asian groups). The trend is also evident in species more commonly assigned to the genus *Platanthera* in boreal species such as *P. chorisiana*. In addition, alteration between paedomorphosis and peramorphosis would explain the evolutionary lability of spur length evident in the group. The green flowers of many species of *Platanthera* could also be ascribed to retention of juvenile features (i.e. production of chlorophyll) in the mature flower.

Among the putatively adaptive floral characters, variations in spur length, caudicle length and viscidial presentation have attracted greatest attention from evolutionary biologists. This emphasis is predicated on the assumption that these features are under the strongest selection pressure, since they are the characters that, together with scent, are considered most likely to determine (or perhaps be determined by) pollinator preference (Luer, 1975; Nilsson, 1983, 1985; Catling and Catling, 1991; Hapeman and Inoue, 1997; Ackerman and Morgan, 2002; Sheviak, 2002). Work is underway to explore the comparative genetics of spur development in Orchidinae (Box *et al.*, 2008; unpubl. res.), but studies of gynostemium development are also highly desirable, particularly if the key genes underpinning the allometric shifts in viscidial placement can be identified. Similarly, the apparent reversions to plesiomorphic ovoid tubers in the former genera *Diphylax* and *Piperia* merit evolutionary–developmental examination, to determine whether they are homologous at the genetic level with those in genera such as *Orchis s.s.*

Compared with some other genera of Orchidinae (e.g. *Dactylorhiza*: Pillon *et al.*, 2007), chromosomal changes appear to have played a limited role in the diversification of the *Platanthera* clade. Although Brandham (1999) listed a wide range of chromosome counts in *Platanthera*, Pridgeon *et al.* (1997) critically appraised those reports and found that, for the range of species sequenced by them, all of the reliable reports gave only the plesiomorphic condition for Orchidinae, $2n = 42$, across the *Platanthera* clade. To the best of our knowledge, only one relevant count has been made in the subsequent decade, specifically $2n = 42$ for *Galearis diantha* (Luo, 2004). The notable exception to this chromosomal consistency is *Platanthera* section *Limnorchis*, where polyploidy apparently occurs frequently. Webb (1980) reported that the Icelandic *P. hyperborea* is tetraploid ($2n = 84$) and the Nordic–Siberian *P. oligantha* (= *P. obtusata* subsp. *oligantha*) is hexaploid ($2n = 126$). In North America, the origins of the allotetraploid *P. huronensis* (cf. Tanaka and Kanemoto, 1984; Sheviak and Bracht, 1998) have been traced to allopolyploidy events involving hybridization between the widespread, moderately closely or closely related diploid species *P. dilatata* and *P. aquilonis* (Fig. 1). At least two origins of *P. huronensis* with the opposite maternal and paternal parents have been inferred (Wallace, 2003, 2004). Although *P. aquilonis* is a facultative autogam, it continues to hybridize occasionally with *P. dilatata* (Wallace, 2006).

More broadly, reports of hybridization among *Platanthera* spp. are spread sporadically across the taxonomic sections, but the reports are uncommon and evidence is often equivocal. Co-evolutionists convinced of the effective pre-zygotic isolation of *Platanthera* spp. might argue that this pattern accurately reflects gene flow among the species. However, sceptics might merely argue that the relatively simple and conservative morphology of the flowers makes morphological identification of hybrids extremely challenging (Bateman and Denholm, 1983; Bateman and Sexton, 2008; Bateman *et al.*, 2009). There have been no credible records of hybridization among members of the three genus-level groupings within the *Platanthera* clade *s.l.* (i.e. the *Pseudorchis, Galearis* and *Platanthera* clades in Fig. 1). This suggests that the levels of genetic disparity render such crosses inviable, reinforcing the validity of the recircumscribed genera.

We believe that understanding of the phylogeny of the *Platanthera* clade has reached a critical threshold. As noted by Hapeman and Inoue (1997), the genus *Platanthera* alone has the potential to provide an exceptional case study in evolutionary diversification and biogeographic expansion. It combines a considerable degree of floral diversification (traditionally ascribed to plant–pollinator co-evolution: Darwin, 1877; Catling and Catling, 1991; Wood and Neiland, 2001*c*) with lesser degrees of vegetative diversification and considerable degrees of sequence divergence with lesser degrees of chromosomal change. Of equal potential value is comparison with the two other groups within the *Platanthera* clade, namely *Galearis–Neolindleya* and *Pseudorchis*. The three groups are readily distinguished by floral characters and, in the case of *Galearis–Neolindleya*, by vegetative characters. Moreover, they provide valuable contrasts in the reward given to pollinators, which has been much discussed in the recent literature (cf. van der Cingel, 1995; Neiland and Wilcock, 1998; Cozzolino and Widmer, 2005; Tremblay *et al.*, 2005). Specifically, substantial nectar is offered by *Platanthera* and limited nectar by *Pseudorchis* and at least one Chinese species of *Galearis* (Yu and Luo, unpubl. res.), but no nectar is provided by the remainder of the food-deceptive *Galearis–Neolindleya* group.

In addition, all three major clades have circumpolar distributions, a pattern broken only by the absence of *Galearis s.l.* from Europe. This makes it especially difficult to infer the geographic origin of the entire *Pseudorchis–Neolindleya–Galaearis–Platanthera* clade. Nonetheless, *Platanthera* and *Galearis–Neolindleya* constitute an excellent biogeographic comparison, given that both clades appear to have originated and diversified at the genus level in East Asia. *Neolindleya* remained endemic to that region (Efimov *et al.*, 2009). *Galearis* reached North America (or could have originated there: Fig. 1), but it failed to undergo substantial speciation on that continent. In contrast, the present tree and that of Hapeman and Inoue (1997) both imply that *Platanthera* reached North America on multiple occasions, with at least some of the lineages subsequently speciating extensively. There is also equivocal evidence that reverse migrations toward East Asia occurred in *Platanthera* sections *Limnorchis* and *Platanthera*. Only one lineage within *Platanthera* section *Platanthera*, the *P. bifolia* group, spread westwards from Asia into Europe, apparently showing modest subsequent morphological divergence but remarkably little detectable molecular diversification (Bateman *et al.*, 2009).

Further substantial progress in understanding evolution and migration in the group via phylogenetic reconstruction will require molecular analysis of all extant species and sequencing of additional nuclear and plastid regions, in the hope of clarifying the complex morphological and biogeographical patterns and improving the statistical rigour of critical nodes, particularly those separating the major species groups. Our incorporation of *Aceratorchis* and *Chondradenia* into *Galearis* and of *Piperia, Diphylax* and *Tsaiorchis* into *Platanthera* (admittedly without molecular support in the cases of the monotypic genera *Aceratorchis* and *Tsaiorchis*, and of the potentially monotypic *Chondradenia*) demonstrates that species comparison should not be confined to pre-delimited genera, most notably by illustrating the relative frequency with which taxa can shift from expected positions in the outgroup to better supported positions in the ingroup.

The relationships among the three major groups within the *Platanthera* clade, and among the eight or more major groups within the genus *Platanthera* itself (Fig. 1), are crucial to interpreting both character change and biogeography. Most notably, Hapeman and Inoue (1997) found the earliest divergent group within *Platanthera* to be the wholly Asian section *Tulotis*, whereas we found it to be the circumpolar section *Limnorchis* (as did R. Lauri, unpubl. res.). These contrasting placements lead to substantially different polarities of both character change and geographical migration. Unfortunately, at present, neither hypothesis of relationship is well supported. A collaborative effort is underway to remedy this situation.

Opportunities now exist to explore simultaneously the morphometrics, population genetics, reproductive biology and autecology of narrowly defined species groups within the *Platanthera* clade. Recent studies combining two or more of these approaches have shown much promise (cf. Bateman, 2001; Wallace, 2002, 2003, 2004, 2006; Holzinger and Wallace, 2004; Little *et al.*, 2005; Lauri, 2007; Bateman *et al.*, 2009). They also suggest that most members of the group have not been studied in sufficient detail, and hence would benefit from rigorous monographic treatment.

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APPENDIX: SAMPLES YIELDING ITS SEQUENCES

Sequence sources from previous publications: 1 = Hapeman and Inoue (1997); 2 = Pridgeon *et al.* (1997; also Bateman *et al.*, 1997); 3 = Bateman *et al.* (2003); 4 = Szalanski *et al.* (2001). * = incorrectly named as *P. hyberborea viridis* by Bateman *et al.* (2003). Collection numbers: EF and AF, GenBank; K, RBG Kew database; B, Bateman collection; L, Lauri collection. Parentheses indicate sequences that are identical to those generated by other listed accessions and so were excluded from parsimony analysis.

Number	Pub	Taxon	Locality	Collector(s)
Related genera (7)				
Ksn	3	*Pseudorchis straminea*	?Sweden	Liden
B63	2, 3	*Pseudorchis albida*	Balvattan Hill, SE Aviemore, Rothiemurchus, C Scotland	R. Bateman
B578	3	*Neolindleya camtschatica*	Ullung Island, Korea	Y. N. Lee
K850	3	*Galearis (Amerorchis) rotundifolia*	Palmerston North, Lanark, Ottawa, Canada	?

Continued

A<small>PPENDIX</small> *Continued*

Number	Pub	Taxon	Locality	Collector(s)
Ksn	2, 3	*Galearis spectabilis*	USA	Davis
Ksn	2, 3	*Galearis cyclochila*	?Japan	K. Inoue
Ksn	3	*Galearis diantha*	China	Y. B. Luo
North America (18)				
B1559		*Platanthera stricta 'gracilis'* [Limnorchis]	Mt Lassen, E Redding, N California, USA	R. Bateman
B1558		*Platanthera dilatata* cf. *leucostachys* 1 [Limnorchis]	N Mt Lassen, E Redding, N California, USA	R. Bateman
EF025518		*Platanthera dilatata leucostachys* 2 [Limnorchis]	USA?	L. Wallace
B1092		*Platanthera dilatata dilatata* 1 [Limnorchis]	Lr Supreme Trail, E Alta, Snowbird, NE Utah, USA	R. Bateman
EF025524		*Platanthera dilatata dilatata* 2 [Limnorchis]	USA?	L. Wallace
EF025521		*Platanthera dilatata albiflora* [Limnorchis]	USA?	L. Wallace
EF025530		*Platanthera* cf. *aquilonis* [Limnorchis]	USA?	L. Wallace
Lsn		*Platanthera* cf. *aquilonis* [Limnorchis]	Alaska, USA	R. Lauri
B486		*Platanthera* cf. *limosa* [Limnorchis]	W Fork Trail, Oak Creek Canyon, Sedona, Arizona, USA	R. Bateman
B1091		*Platanthera sparsifolia s.s.* [Limnorchis]	Mossy Cave Trail, NE Ruby's Inn, Bryce, S Utah, USA	R. Bateman
Ksn	1, 2, 3	*Platanthera grandiflora* [Lacera]	USA	V. Albert
K9161		*Platanthera peramoena* [Lacera]	USA	D. Nickrent
AF301445	4	*Platanthera praeclara* [Lacera]	USA?	?
Lsn		*Platanthera orbiculata* [Lysias]	USA	R. Lauri
K930	3	*Platanthera* (*Piperia*) *unalascensis*	California, USA	J. Hapeman
Bsn	3	*Platanthera* (*Piperia*) *colemanii*	California, USA	W. Temple
Bsn	3	*Platanthera* (*Piperia*) *elongata*	California, USA	W. Temple
Lsn		*Platanthera chorisiana*	USA?	R. Lauri
Southeast Asia (10 > 8)				
Ksn	1, 2, 3	*Platanthera* (*hyperborea*) *viridiflora** [Limnorchis]	?Japan	K. Inoue
Ksn	1, 2, 3	*Platanthera sonoharai* [Tulotis]	?Japan	K. Inoue
K13087		*Platanthera mandarinorum*	China	Y. B. Luo
Ksn	1, 2, 3	*Platanthera florentia*	?Japan	K. Inoue
K8048		*Platanthera bakeriana*	China	Y. B. Luo
Ksn		*Platanthera* (*Diphylax*) *urceolata*	China	Y. B. Luo
Ksn		*Platanthera* (*Diphylax*) sp.	China	Y. B. Luo
(K13038)		*Platanthera finetiana*	China	Y. B. Luo
(K13073)		*Platanthera metabifolia*	China	Y. B. Luo
K13071		*Platanthera chlorantha*	China	Y. B. Luo
Europe (8 > 7)				
H3054		*Platanthera oligantha* [Lysiella]	N Norway	M. Hedrén
B787		*Platanthera chlorantha*	Homefield Wood, Medmenham, Bucks, UK	R. Bateman
B1033		*Platanthera bifolia*	Morgans Hill, SE Calne, Wilts, UK	R. Bateman
(B1398)		*Platanthera holmboei*	Copse N crossroads, Mandria, Troodos, C Cyprus	R. Bateman
B2193		*Platanthera micrantha* 1	Lagoa di Canario, San Miguel, Azores	M. Moura
B2194		*Platanthera micrantha* 2	Lagoa di Canario, San Miguel, Azores	M. Moura
B2195		*Platanthera azorica* 1	Lagoa di Fogo, San Miguel, Azores	M. Moura
B2196		*Platanthera azorica* 2	Lagoa di Fogo, San Miguel, Azores	M. Moura