KEW BULLETIN (2018) 73: 4
DOI 10.1007/S12225-017-9728-Z

ISSN: 0075-5974 (print)
ISSN: 1874-933X (electronic)

Clarified relationship between *Dactylorhiza viridis* and *Dactylorhiza iberica* renders obsolete the former genus *Coeloglossum* (Orchidaceae: Orchidinae)

Richard M. Bateman[1] & Paula J. Rudall[1]

Summary. Two decades have passed since DNA evidence first demonstrated an intimate relationship between the circumboreal species *'Coeloglossum' viride* and the temperate Eurasian genus *Dactylorhiza s.s.* Most subsequent molecular phylogenies showed *'C.' viride* to diverge *after* the *D. incarnata* group. The law of monophyly therefore dictated inclusion in *Dactylorhiza* of *'C.' viride*, irrespective of its undeniably distinctive morphology. Those orchid enthusiasts still determinedly seeking reasons for retaining the genus *Coeloglossum* have often used as a justification the one published molecular study that suggested that *D. viridis* diverged earlier than *D. incarnata*. Interestingly, these respective phylogenetic positions are supported by recent data-rich studies based on next-generation sequencing. However, recent DNA phylogenies also show that *D. iberica* — long regarded as morphologically distinct but nonetheless universally accepted as belonging within the genus — diverged penecontemporaneously with *D. viridis*. Thus, in order to justify maintaining *'Coeloglossum'* as a separate monotypic genus it would also be necessary to transfer *D. iberica* to a new monotypic genus, thus recognising two genera that are not only monotypic but also show only modest molecular divergence from the remaining dactylorchids. Examining in greater detail the morphology and micromorphology of *D. viridis* and *D. iberica*, we show that both species possess multiple morphological character states that are unique within the genus *Dactylorhiza*, but argue that greater phenotypic disparity is commonly the case in early-divergent lineages per se. Review of previous publications discussing *D. iberica* revealed little knowledge of its autecology, and contradictory DNA-based inferences that can be traced back to just two original specimens. We also suggest that morphological and molecular variation within both species has been under-estimated and under-explored.

Key Words. Evolutionary tree, genus delimitation, monophyly, morphology, next-generation sequencing, nuclear ribosomal ITS, phylogeny, species delimitation.

Introduction

During the last two decades it has become *de rigueur* to circumscribe supraspecific taxa by applying the principle of monophyly (groups of species consisting of all the descendant species of a hypothetical shared ancestor) to evolutionary matrices that consist of DNA sequences derived from specific 'candidate' genes. The innate charisma of orchids has placed them at the forefront of 'new wave' systematics. However, classifications derived using this approach often contradict previous classifications that were typically based on perceived morphological similarity. Controversy has rarely been greater than that surrounding monophyletic classifications of north-temperate orchids following publication of the initial genus-level circumscriptions by Pridgeon *et al.* (1997) and Bateman *et al.* (1997).

Acknowledging the previous 12 years of (sometimes contentious) debate, Bateman (2009, his Table 1) tabulated taxonomic treatments of European orchids published between 2001 and 2008 in order to illustrate the fact that no authors residing outside Britain had yet accepted the re-circumscriptions of all of the affected genera. Most authors simply preferred either to wholly reject the contribution of DNA studies to orchid classification on the grounds that they were in some way unintuitive or to accept only those portions of the DNA-based classification they found intuitively acceptable. Few of these authors have made serious attempts to explain their reasoning in either conceptual or practical terms. Interestingly, the least popular genus-level re-circumscriptions have proven to be the incorporations of (a) all species of *Listera* into *Neottia* (thereby circumscribing a genus that combines autotrophs with mycoheterotrophs) and (b) the monotypic *Coeloglossum* into *Dactylorhiza* as *D. viridis* (L.) R. M. Bateman, Pridgeon & M. W. Chase (a decision reviewed scientifically by Bateman 2009 and nomenclaturally by Cribb & Chase 2001).

Accepted for publication 15 November 2017. Published online 23 December 2017

[1] Jodrell Laboratory, Royal Botanic Gardens Kew, Richmond, Surrey, TW9 3DS, UK. e-mail: r.bateman@kew.org

Table 1. Morphological distinctions identified by Devos *et al.* (2006b) as separating '*Coeloglossum*' *viride* from other species of *Dactylorhiza*, reproduced verbatim from their Table 1 but with footnote annotations provided by the present authors.

Character	*Coeloglossum*	*Dactylorhiza*
Flowers: general colour	Green, sometimes reddish or brownish	White, pink, purple, yellow
Perianth arrangement	Lateral sepals included in protective hood	Lateral sepals spread (exception: *D. iberica*)[1]
Lip shape	Elongated, with bifid apex and small tooth in indentation	Generally three-lobed, with wide lateral lobes and variable median lobe; seldom undivided
	Oblong, with parallel lateral margins	Elliptical to rhomboidal[2]
Lip length vs width	Much longer than wide	Wider than long or equal[3]
Lip markings	None	Generally red to purple spots, lines and/or loops; seldom no markings
Spur shape and aspect	Roughly spherical; shiny	Cylindrical to conical, rarely sac-shaped; matte
Spur length	Much shorter than ovary	Much longer than, to roughly equal to, ovary[4]
Nectar production	Spur nectariferous	No nectar
Pollinia	Strongly divergent	Parallel, close to each other
Viscidia	Naked, in rudimentary bursicles	Joined together in a well-formed bursicle

Present authors' annotations: [1] true of Greek plants, less so of Turkish plants; [2] except *D. iberica*, elongate deltoid; [3] except *D. iberica*, longer than wide; [4] except *D. iberica*, shorter than ovary.

In the case of re-assigning *Coeloglossum viride* (Frog Orchid: Fig. 1A) to *Dactylorhiza*, the most direct and well-argued challenge was issued by Devos *et al.* (2006b; re-iterated and elaborated by Tyteca & Klein 2008, 2010). Their thesis boiled down to two main arguments (Table 1). Firstly, they listed (for the most part accurately) several morphological characters in which the Frog Orchid deviates substantially from other European species within the genus *Dactyorhiza*. Secondly, after having generated their own DNA tree, they regarded as equivocal the previous DNA evidence presented by several authors (notably Bateman *et al.* 2003) for the Frog Orchid being nested evolutionarily within the previously accepted circumscription of the genus *Dactylorhiza*. More specifically, DNA trees produced by several research groups sequencing regions of both the nuclear (notably nuclear ribosomal Internal Transcribed Spacer: nrITS) and plastid (e.g. *rpl16*) genomes had all suggested (albeit tentatively) that, during the evolution of *Dactylorhiza*, the *D. incarnata* group (early marsh-orchids: Fig. 1B) had originated **before** *D. viridis* (Frog Orchid: Fig. 1A).

However, most of these molecular studies were conducted in the absence of several species of *Dactylorhiza*, in part because sampling focused on European rather than Asiatic species. Here, we argue that one of the species that was omitted from most of these studies and was represented inadequately in the remainder is crucial in determining not only its phylogenetic position and genus-level assignment but also that of *D. viridis*. That species is *Dactylorhiza iberica* (M. Bieb ex Willd.) Soó (Fig. 1C), a comparatively late-flowering inhabitant of moist soils in the afforested mountains of Greece, Cyprus, Crimea, Turkey and the Caucasus, extending as far east as Iran (Kreutz 1998; Petrou *et al.* 2011; Antonopoulos 2015). Although well-circumscribed morphologically and universally accepted as a full species, comparatively little is known abut this species (indeed, its unfortunate species epithet means that many botanists mistakenly believe that it occupies the Iberian Peninsula rather than the region of Georgia after which it was named by von Bieberstein in Willdenow 1805). Several distinct morphological features of *D. iberica* have caused some previous authors to argue that it was most likely primitive within the genus. However, both *D. viridis* and especially *D. iberica* have received less research attention than other, more derived taxa within the genus that have become a well-known model system for the study of polyploidy events (e.g. Hedrén *et al.* 2001; Pillon *et al.* 2007; Paun *et al.* 2010, 2011; Hedrén *et al.* 2011).

Here, we aim to draw more attention to these two phylogenetically pivotal species, describe in detail their micromorphology, and use accumulated recent knowledge of their morphology and DNA profiles to re-appraise and clarify their much-discussed relationship with the remainder of the genus *Dactylorhiza*. Following the publication of Devos *et al.* (2006b), many authors have sought refuge in the statement that ambiguities in the phylogenetic placement of the Frog Orchid require resolution, and until that resolution is achieved, recognition of the monotypic genus '*Coeloglossum*' should continue. We here demonstrate that knowledge of *D. viridis* exceeds that of most other plant species whereas ambiguities in its phylogenetic placement do not. Our aim is to sound the long overdue death-knell for the former genus *Coeloglossum* (Cribb & Chase 2001; Bateman *et al.* 2003; Bateman 2009, 2012a).

Materials and Methods

For the last three years we have pursued with Ian Denholm an *in situ* morphometric study of popula-

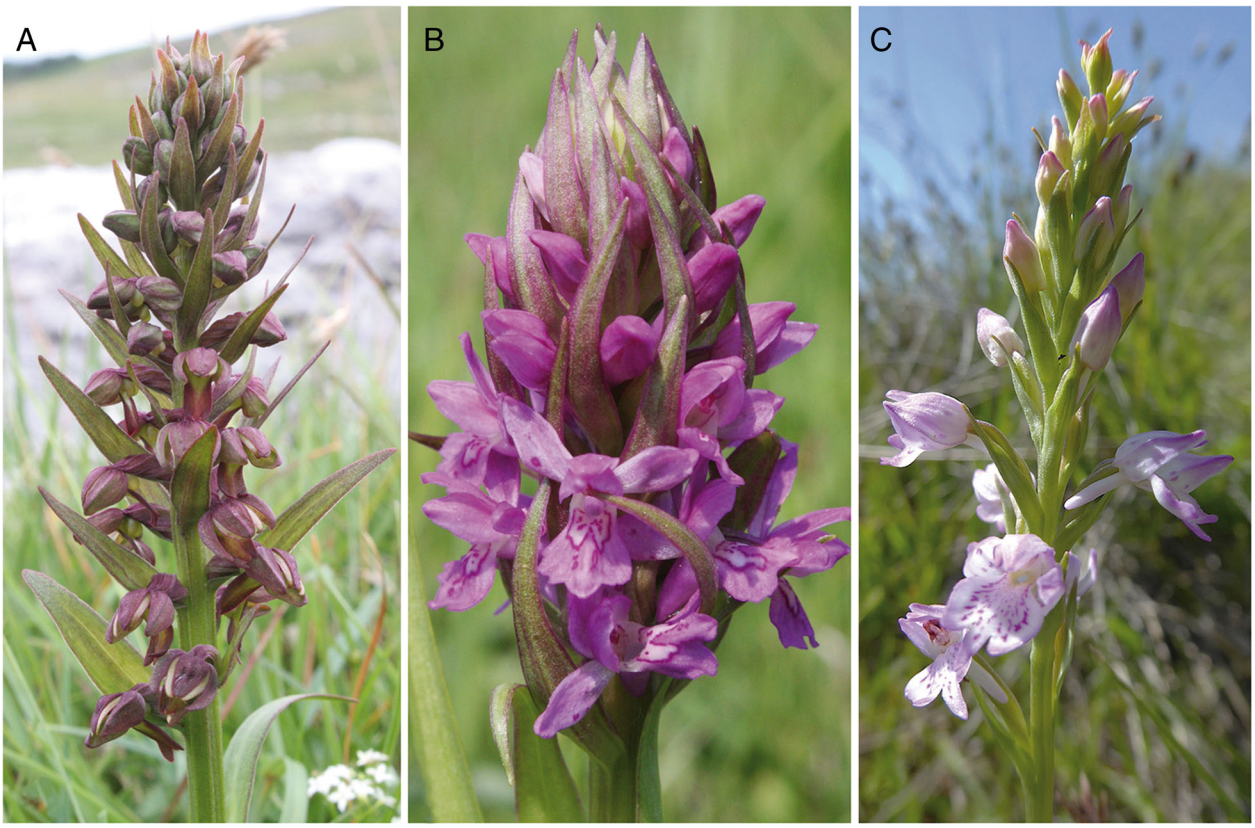

Fig. 1. Flowers of the three primary contenders for the position of earliest-divergent lineage within the genus *Dactylorhiza*. **A** *Dactylorhiza viridis*. Ingleborough Hill, Yorkshire, U.K. (290 m), 22 June 2014. **B** *Dactylorhiza incarnata incarnata*. Dabas, SE Budapest, Hungary (95 m), 11 May 2013. **C** *Dactylorhiza iberica*. Kallithea, Epiros, N Greece (960 m), 13 June 2017. PHOTOS: **A, B** RICHARD BATEMAN, **C** PAULA RUDALL.

tions of *Dactylorhiza viridis*, as a result of which we had available numerous well-characterised DNA specimens sampled from known habitats, and a smaller number of flower samples preserved in alcohol. Our field knowledge of *D. iberica* derives primarily from field-work in the Pindos Mountains of northern Greece, though alcohol-preserved flowers of this species from several localities and three countries were previously available in the spirit collections at K.

The following specimens were examined for scanning electron microscopy (SEM): *Dactylorhiza iberica*, K 27933 (*Davis & Hedge* 30117, Turkey 1957); *D. viridis*, K 5881 (*Sayers* 09/10/11, Austria 1961); *D. viridis*, K 22537 (*Hunt* 1375, UK 1961); *D. viridis*, K 25974 (Jeans, UK 1963); *D. viridis*, K 1396 (collector unknown).

Preparation for SEM involved selecting flowers from each preserved inflorescence for dehydration through an alcohol series to 100% ethanol. They were then stabilised using an Autosamdri 815B critical-point drier, mounted onto stubs using double-sided adhesive tape, coated with platinum using an Emtech K550X sputter-coater, and examined under a Hitachi cold-field emission SEM S-4700-II at 2 kV. The resulting images were recorded digitally for subsequent enhancement in Adobe Photoshop.

Results

Dactylorhiza viridis

In the case of *Dactylorhiza viridis*, our aim is simply to supplement and extend observations previously made through both scanning electron and light microscopy by our own research group, including characterisation of pre-anthesis ontogenetic stages (Box *et al.* 2008; Bell *et al.* 2009).

Dactylorhiza viridis is on average smaller-bodied than any other species in the genus, although proportionately, it does not differ radically from the other species in vegetative features. The oblong, parallel-sided labellum reaches 4.5 – 6 mm, ending in a small, tooth-like central lobe that is exceeded by the lateral lobes, coloured anything from uniform yellowish-green through to purplish-brown paling toward the spur entrance; it cannot be confused with that of any other dactylorchid (Figs. 1A, 2A – C). Nor can the spur itself, which is a near-globose downward-oriented sac 1.5 – 2 mm in diameter, contrasting with the larger cylindrical-conical spurs that characterise the remainder of the genus. Details of the column are difficult to resolve by eye, necessitating microscopic examination. Overarching the column are the ovate lateral petals

and sepals, connivent into a compact hood that extends outward for 5 – 6 mm.

SEM study reveals that the adaxial epidermis of the labellum is fairly uniform and composed of papillate cells, somewhat elongate longitudinally (c. 55 × 30 µm) and more subdued above the mid-vein and adjacent to the highly constricted, near-circular labellar spur entrance (Fig. 2A – C, E) (see also Box *et al.* 2008; Claessens & Kleynen 2011). As befits a non-secretory spur, its inner (adaxial) epidermis lacks papillae but does appear to exhibit vermiform to reticulate cuticular texture on equi-dimensional epidermal cells c. 30 µm in diameter (see also Bell *et al.* 2009). Cells of the adaxial labellar surface are gently domed away from the mid-vein (Fig. 2E). Just below (i.e. distal to) the spur is a distinct elongate boss, located midway between two raised lateral ridges, which work together to delimit two channels that extend downwards from a pair of lateral recesses (Fig. 2A, C). Domed papillae located in the recesses are responsible for secreting modest volumes of nectar that, under the influence of gravity, dribble downward along the channels. The channels are located immediately below the discoid lateral auricles, which also appear to secrete nectar. Current evidence suggests that these small quantities of nectar are unique to this species within the genus *Dactylorhiza*, and are reputedly associated with a slight honey-like scent. The stigma is relatively undifferentiated, apparently simply consisting of a secretory oval region in the roof of the spur entrance immediately below the rostellum; secretory residues persist under the SEM (Fig. 2F). In many flowers coarse massulae derived from pollinia adhere tightly to, and usually obscure, this surface (Fig. 3A, B).

The gynostemium is compact and approximately equi-dimensional at c. 1.5 mm (Fig. 3A – E). The cowl-like connective is well-developed and composed of slightly domed, equi-dimensional cells c. 40 µm in diameter. The thecae are adjacent apically, but diverge downward toward the spur entrance, such that the unusual paired, delicate bursicles (so delicate that the viscidia are described as naked by some observers) are comparatively well-separated by the long, shelf-like rostellum that extends laterally into modest-sized auricles (Fig. 3A – D). A cavernous depression immediately below the rostellum circumscribes a slightly vertically elongate, oval stigmatic surface. Each of the two c. 0.7 mm-long pollinaria consists of a pyriform pollinium composed of several distinct massulae, linked via a comparatively short caudicle to a fairly substantial, discoid viscidium c. 150 µm in diameter (Figs. 2C, 3C, E, F). Cells of the seed testa are unusual within the genus in being smooth walled, sharing this iteratively evolving feature with *Dactylorhiza incarnata* (L.) Soó and the genus *Gymnadenia* s.l. (Gamarra *et al.* 2015).

Dactylorhiza iberica

In contrast with *Dactylorhiza viridis*, there is a paucity of accurate, detailed descriptions of *D. iberica*. It typically occurs as a more slender plant than most other dactylorchids, its narrow erect leaves more akin to those of *Dactylorhiza*'s sister genus, *Gymnadenia s.l.*

The flowers of *Dactylorhiza iberica* broadly resemble those of *D. viridis* in shape (Fig. 1C). All perianth segments other than the labellum are similarly connivent to form a (sometimes loose) 7 – 9 mm hood, and the labellum is longer than broad and shallowly three-lobed, 7 – 9 × 5 – 7 mm (Figs. 1C, 4A). The central lobe is similarly small and tooth-like, although it tends to equal or project slightly beyond the lateral lobes. As in *D. viridis*, the labellum is more likely to be concave than convex, but here it narrows to a more constricted base. The spur is slender (c. 1 mm), cylindrical, slightly down-curved and approximately half the length of the ovary (c. 5 mm); it has parallels elsewhere in the genus (most notably in *D. maculata*). The flowers occur in various shades of pink, and the central region of the labellum bears darker spots, typically longitudinally elongate and delineating crude longitudinal arrays.

To the best of our knowledge, flowers of *Dactylorhiza iberica* have not previously been subjected to SEM study (Figs 4, 5). The labellum is thinner and more flexible than that of *D. viridis*. Consequently, the veins are visibly raised in the SEM images; they can easily be traced as they gradually diverge distally until they reach the somewhat irregular labellum margin (Fig 4A, B). The adaxial epidermis of the labellum consists (other than in the throat) of polygonal cells c. 65 µm in diameter that extend outward into comparatively long semi-rigid columnar trichomes (Fig. 4A – C). Each trichome gradually narrows to c. 10 µm in diameter before terminating in an apparently glandular globose apex c. 18 µm in diameter. These putative glands are 100 – 130 µm long, reaching 150 µm on the mid-vein but not being noticeably longer in the purple-spotted regions (as with other spotted dactylorchids but in contrast with, for example, anthropomorphic *Orchis* species). Only in the vicinity of the spur entrance do the epidermal cells grade into more conventional lower-relief papillae that resemble cells of the abaxial epidermis (Fig. 4B). The dense carpet of outward projections that characterises most of the labellum surface most closely resembles a rubber bath-mat; it is highly distinctive, having no obvious parallel elsewhere within the genus *Dactylorhiza*.

The spur is thin-walled (Fig. 4D). Both adaxial and abaxial epidermal cells are longitudinally (axially) elongate, c. 40 µm wide and about twice that in length. Those lining the interior of the spur are flat, lacking secretory papillae, but each exhibits several low-relief transverse bars that presumably represent ridges formed in the overlying cuticle (Fig. 4E). The cordate stigma surrounding the spur entrance resembles those of most other dactylorchid species (Fig. 5).

The gynostemium differs considerably from that of *Dactylorhiza viridis*, more closely resembling those of

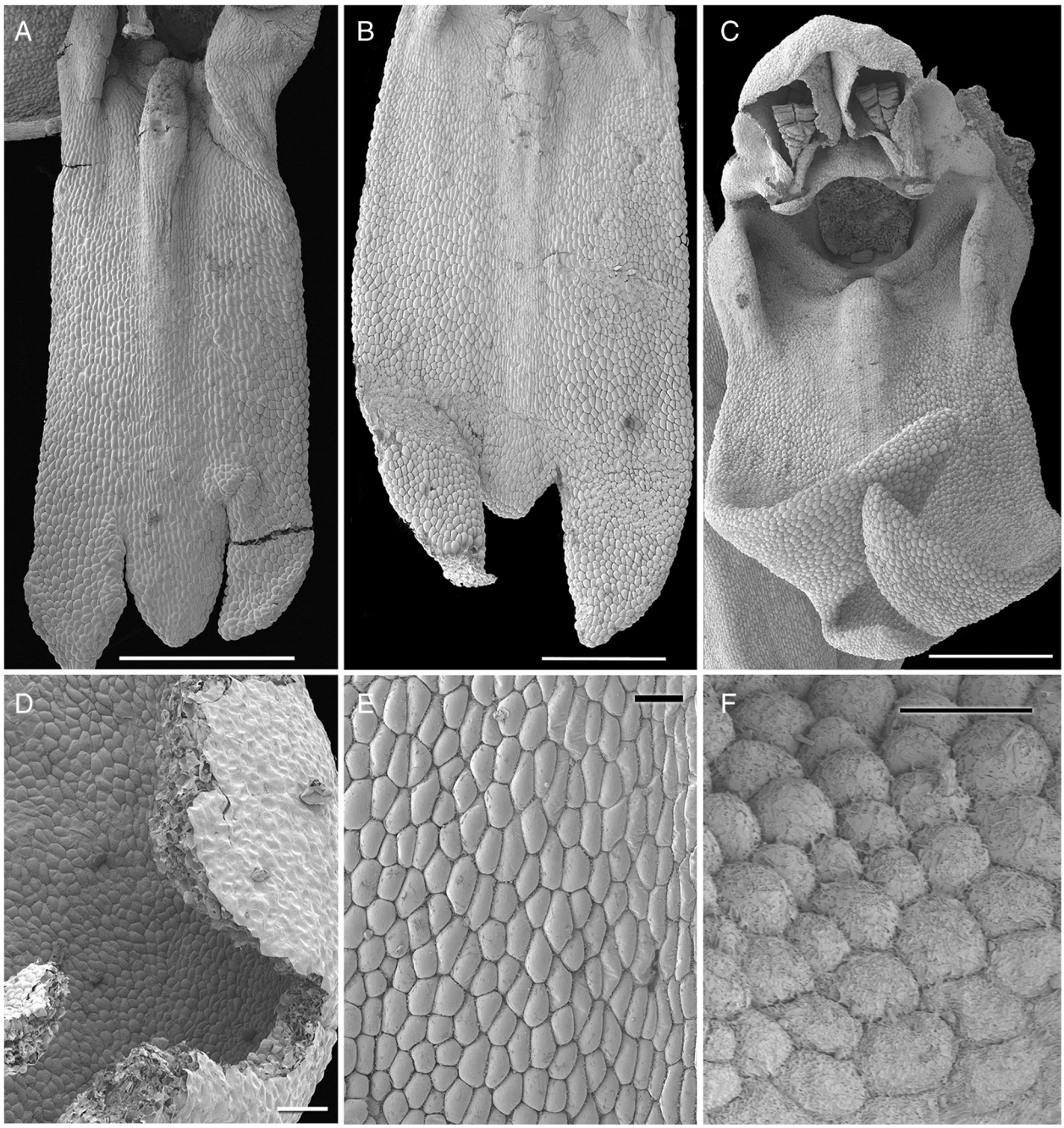

Fig. 2. *Dactylorhiza viridis*, SEMs of flowers. **A, B** entire labellum; **C** entire flower, showing pollinaria and stigma; **D** smooth interior surface of spur; **E** domed cells of labellum adaxial surface; **F** cells at top of labellum, just below stigma, showing desiccated remains of secretions. **A** K 22537; **B, C, E – F** K 25974. Scale bars: **A – C** = 1 mm, **D** = 250 µm, **E** = 100 µm, **F** = 50 µm. PHOTOS: PAULA RUDALL.

other dactylorchids; it is c. 2 × 1.2 mm (Fig. 5). The connective and loculi consist of equi-dimensional cells c. 55 µm in diameter. The loculi resemble those of *D. viridis*, but rather than diverging towards two bursicles separated by a broad, approximately horizontal rostellum, they converge toward a single bursicle that projects c. 1 mm outward beyond the stigma into the aperture formed by the spur entrance, causing the rostellum to become tightly folded vertically (Fig. 5). The sole bursicle is robust, c. 0.5 mm wide and consists of longitudinally elongated cells c. 35 × 20 µm in diameter. Because it is hinged it resembles the toe of a slipper, wholly enclosing the viscidia. In contrast with *D. viridis*, the lateral auricles are so subdued that they are barely recognisable (Fig. 5). The pollinaria are c. 1.5 m long — considerably larger than those of *D. viridis*, and with a longer caudicle in proportion to the club-shaped pollinium (Fig. 5B).

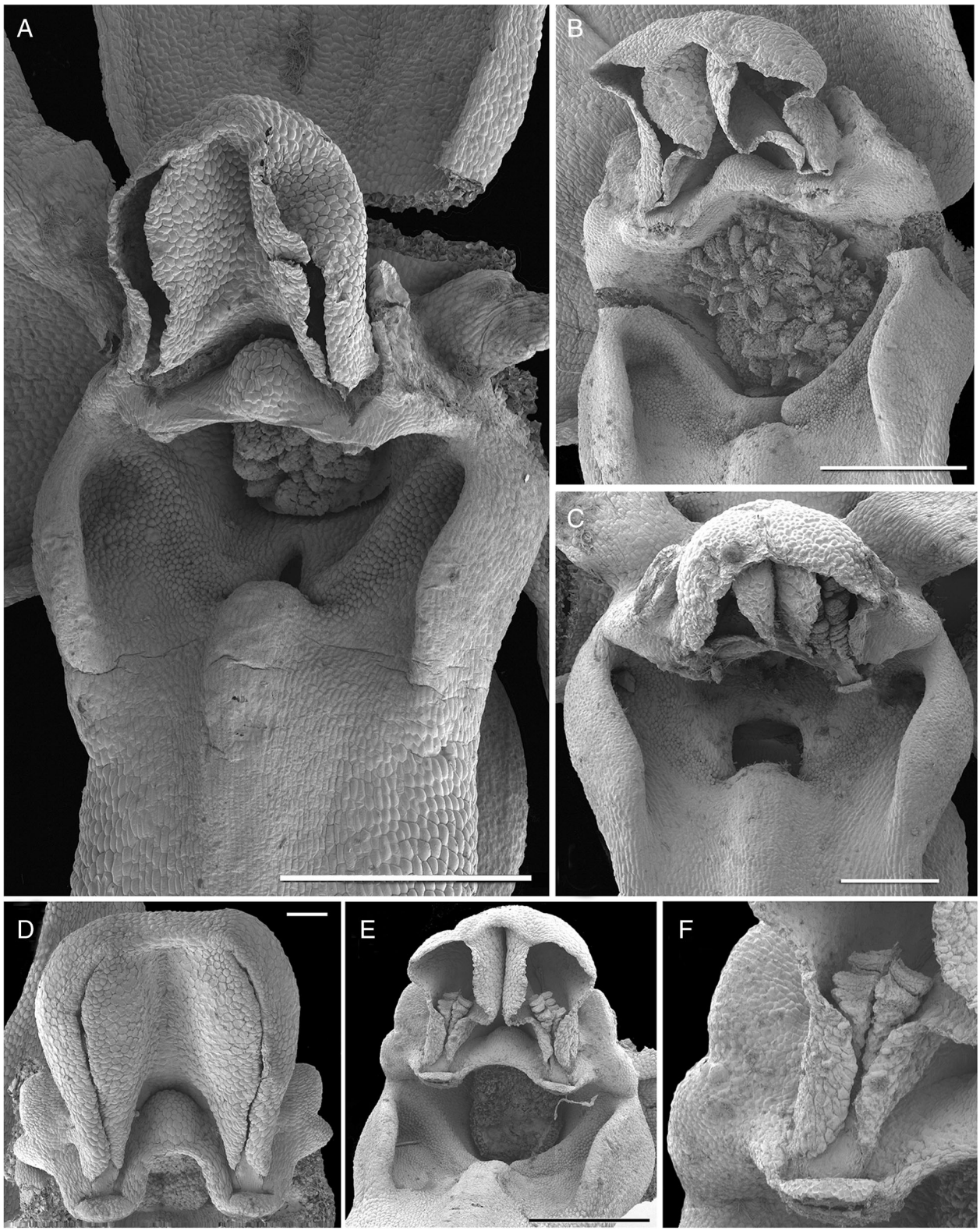

Fig. 3. *Dactylorhiza viridis,* SEMs of gynostemia. **A – C**, **E** open flowers, gynostemium and top of labellum, showing stigma (**F**: detail of anther in E); **D** immature gynostemium of dissected unopened bud. **A** K 5881, **B** K 25974, **C** K 22537, **D – F** K 1396. Scale bars: **A – C**, **E** = 1 mm, **D** = 200 μm, **F** = 100 μm. PHOTOS: PAULA RUDALL.

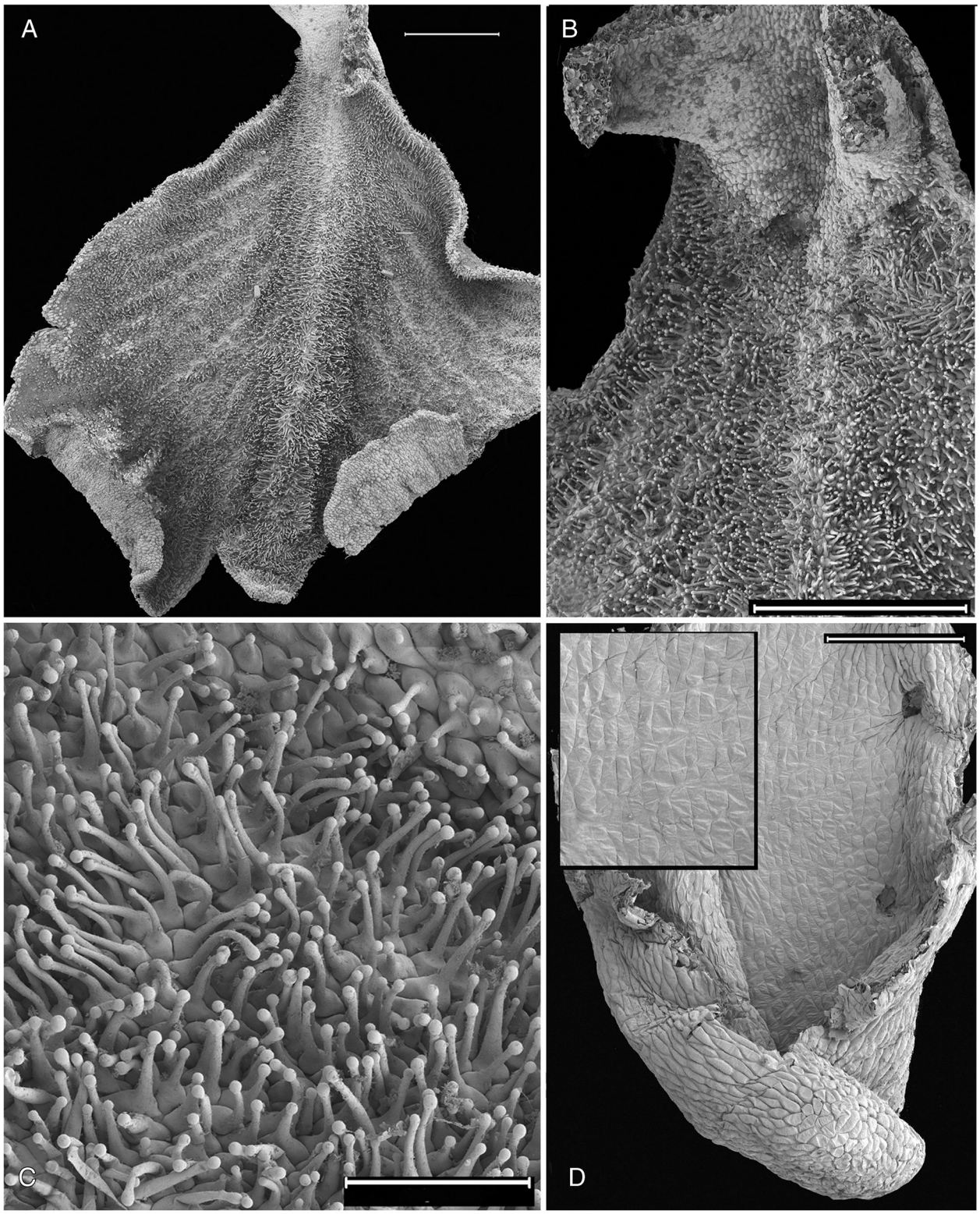

Fig. 4. *Dactylorhiza iberica*, SEMs of labellum. **A** entire labellum; **B** proximal portion of labellum; **C** elongate glandular trichomes on labellum surface; **D** spur interior (inset: detail of smooth surface inside spur). All from K 27993. Scale bars: **A** = 1 mm, **B** = 1 mm, **C** = 250 μm, **D** = 200 μm. PHOTOS: PAULA RUDALL.

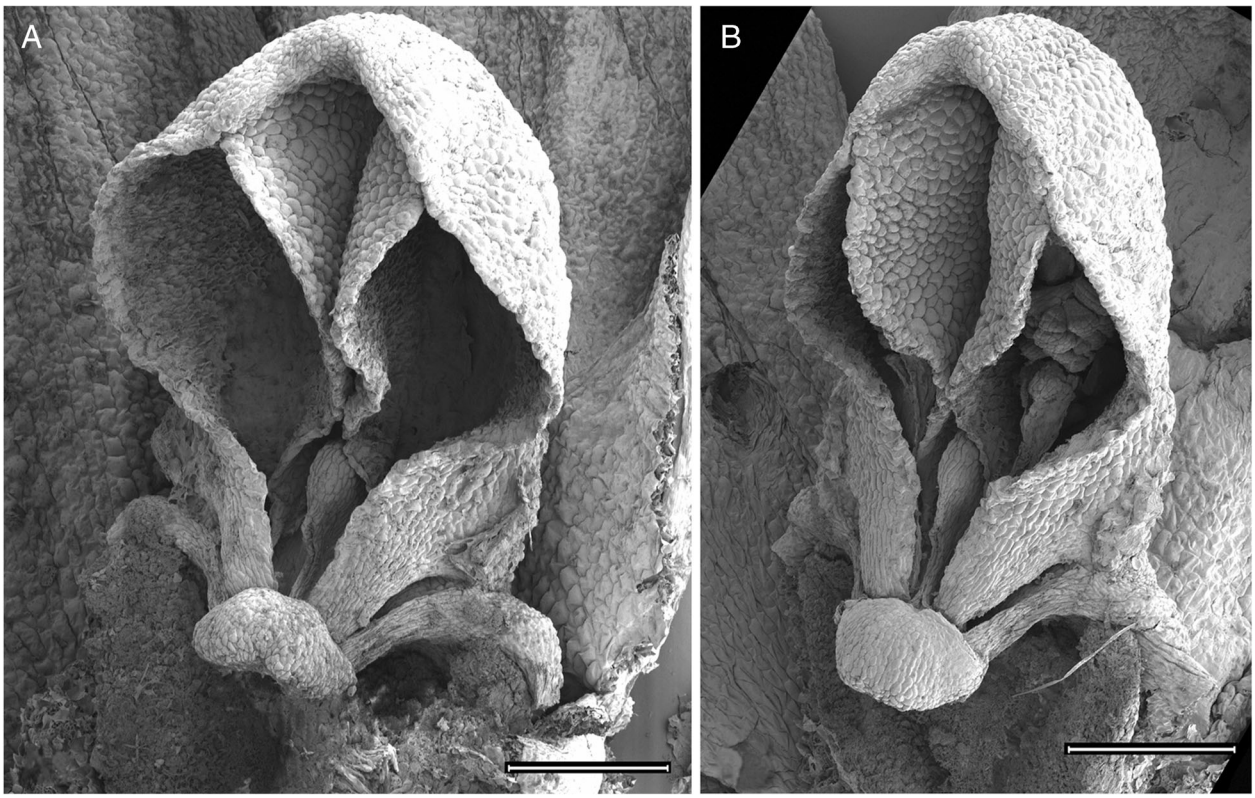

Fig. 5. *Dactylorhiza iberica*, SEMs of gynostemia lacking (**A**) and still retaining (**B**) pollinaria. All from K 27993. Scale bars = 500 μm. PHOTOS: PAULA RUDALL.

Discussion

History of molecular study of *Dactylorhiza iberica* and *D. viridis*: a tangled web

To the best of our knowledge, eight published molecular phylogenetic studies have thus far included at least one DNA sequence (or one AFLP profile in the case of Hedrén *et al.* 2001) that was ostensibly derived from specimens of *Dactylorhiza iberica*. Five of those studies are summarised here in Fig. 6. However, careful examination suggests that most of these studies relied on a single specimen held on the RBG Kew DNA Bank as "*Chase* O-960", which was originally field-collected in the Troodos Mountains of Cyprus by C. Lovell in 1982 and accessioned into the Kew living collection. The first nuclear ribosomal Internal Transcribed Spacer (ITS) sequence generated from this sample attributed to *D. iberica* was published by Pridgeon *et al.* (1997; see also Bateman *et al.* 1997). Surprisingly, the sequence showed a strong similarity to, and appeared to have been derived from, those of *D. foliosa* (Sol. ex Lowe) Soó and *D. maculata* (L.) Soó — a phylogenetic placement later confidently discounted by Bateman *et al.* (2003) as reflecting an erroneous sequence (nonetheless, the original, highly dubious sequence was later employed in the molecular dating analysis of Sramkó *et al.* 2014).

The order of lineage divergence inferred by Pridgeon *et al.* (1997) was first the *incarnata* group, followed by *viridis*, *aristata*, the *romana* group and the remainder. The same basic sequence was found during Bateman *et al.*'s (2003) analysis of a much-expanded ITS dataset (Fig. 6A). Analysis of a sample of *Dactylorhiza iberica* collected in the Pindos Mountains of northern Greece was conducted too late for its inclusion in the published tree; the authors simply reported in the text its position as near-basal within the genus. Identical topologies, differing only in bootstrap support values, were later produced from ITS matrices by Pillon *et al.* (2007), Tang *et al.* (2015) and Bateman *et al.* (in review).

The position in the ITS tree of the Pindos sample, placed tentatively above the *incarnata* group but confidently below *viridis*, was also shown by Bateman & Denholm (2003, Fig, 4). In addition, their Fig. 5 presented the first published plastid (*trnL-F*) data derived from *Dactylorhiza viridis*; the resulting tree, which unfortunately lacked *D. iberica*, showed the *incarnata* group, *viridis* and the *romana* group as being equally probable competitors for the title of earliest-divergent lineage within *Dactylorhiza*. A similarly poorly resolved result was obtained by Hedrén *et al.* (2001) using a different molecular approach — the gene fragmentation technique Amplified Fragment Length Polymorphism (AFLP).

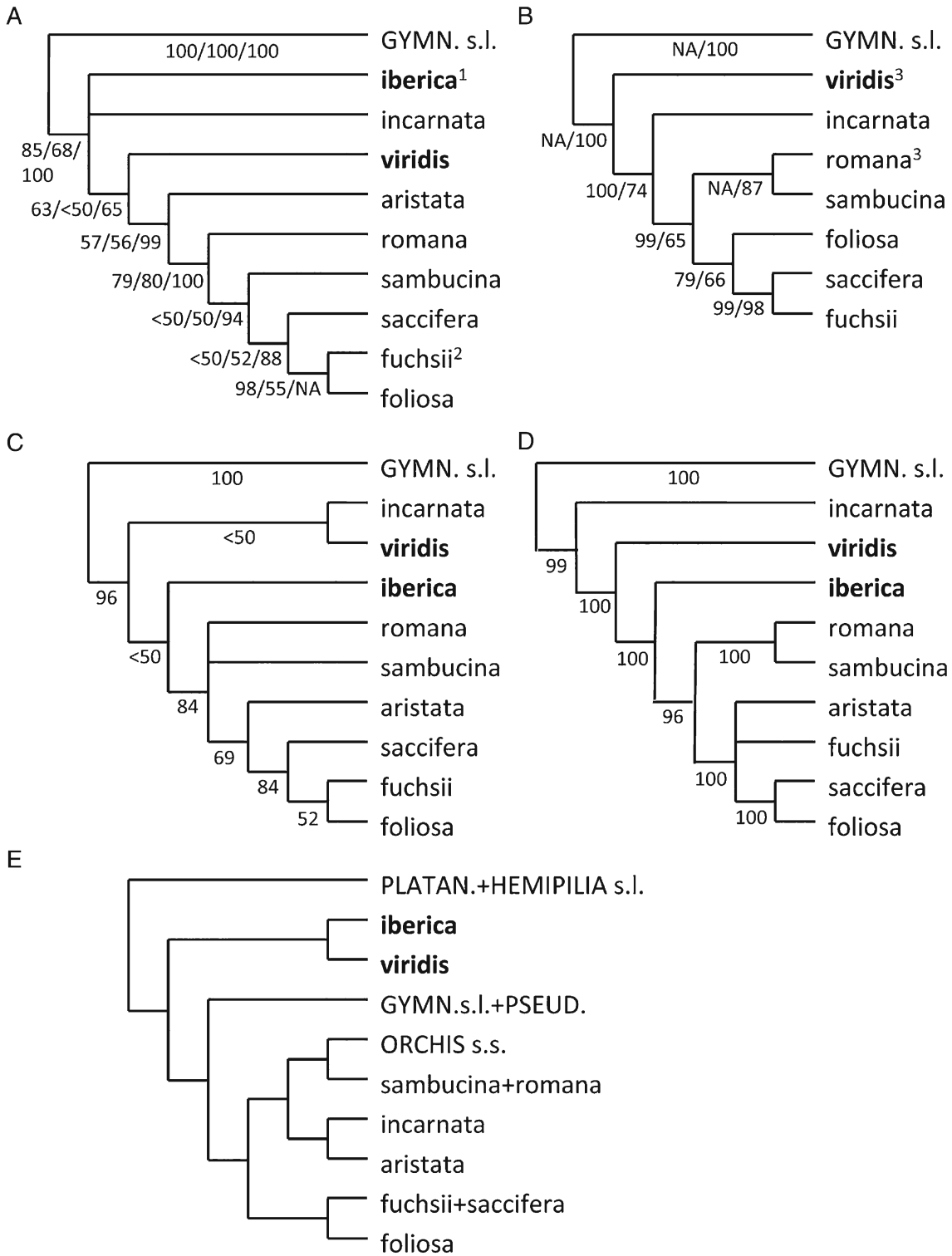

Fig. 6. Outline topologies of several molecular phylogenetic studies that included substantial numbers of *Dactylorhiza* species, highlighting in boldface the variable positions of *D. viridis* and *D. iberica*. **A** nrITS only — Bateman *et al.* (2003), MP, BS; Pillon *et al.* (2007), MP, BS; Tang *et al.* (2015), B, PP. **B** nrITS+nrETS — Devos *et al.* (2006a), ML, BS; Devos *et al.* (2006b), ML, BS. **C** nrITS+*rpl16* — Pillon *et al.* (2006), MP, BS. **D** nrITS+*rpl16*+*coxI* — Inda *et al.* (2012), B, PP. **E** morphology — Bateman *et al.* (in review), MP, all BS <50%. Abbreviations: B Bayesian, ML maximum likelihood, MP maximum parsimony; BS bootstrap, PP posterior probability, [1]position reported in text only by Bateman *et al.* (2003), [2]group absent from Tang *et al.* (2015), [3]group absent from Devos *et al.* (2006a). GYMN *Gymnadenia* s.l., PLATAN *Platanthera*, PSEUD *Pseudorchis*.

Rather than spanning subtribe Orchidinae, the study of Pillon *et al.* (2006; see also Shipunov *et al.* 2004) considered only *Dactylorhiza* and combined nuclear ITS data with the rapidly mutating plastid region *rpl16* in a simultaneous analysis (Fig. 6C). The resulting topology resembled those of earlier studies except in placing *D. viridis* as sister to the *D. incarnata-euxina* group, whereas *D. iberica* (yet again represented by sample 960) was sister to the *D. romana* group, *D. aristata* (Fisch. ex Lindl.) Soó and the remainder. In other words, it became difficult to determine whether *D. viridis* or *D. iberica* was the earliest-divergent dactylorchid. And once again, these inferred relationships attracted disappointingly little statistical support.

A year later, Pillon *et al.* (2007, their Appendix 1) published a considerably expanded dataset that included ten geographically disparate samples of *Dactylorhiza viridis* and two samples of *D. iberica*; sample 960 from Cyprus was finally joined by sample "*Hedrén* 98078" from northwest Turkey. Nonetheless, sample 960 — presumably by now re-sequenced — was once again selected to represent *D. iberica* in their ITS tree. This showed *D. iberica* and the *D. incarnata* group to have equal likelihood of being the earliest-divergent lineage, although the placement of *D. viridis* immediately above these lineages did not attract statistical support (Fig. 6A). The same result was later obtained from an exceptionally well-sampled array of ITS sequences by Tang *et al.* (2015, their Fig. 1). Pillon *et al.* (2007) also analysed microsatellites in the plastid regions *trnS-trnG*, *trnL-F* and *trnL* intron, yielding a good range of plastid haplotypes that proved useful for distinguishing dactylorchid taxa. Unsurprisingly, both *D. viridis* and sample 960 of *D. iberica* proved to have unique and distinct haplotypes, the latter reputedly yielding ribotype XI and haplotype J. What the authors failed to mention in their text (but is evident from their Appendix 1) was the fact that the Turkish specimen 98078 of *D. iberica* had actually yielded ITS ribotype X and plastid haplotype E. Give that both characteristics are typical of *D. incarnata*, this assertion constituted another highly improbable genotyping result attributed to — but perhaps not actually representing — *D. iberica*.

By this point in time, likelihood and Bayesian approaches had replaced parsimony as the most popular approach for building phylogenetic trees. They were used alongside parsimony by Devos *et al.* (2006b) to repeatedly analyse a matrix in which they added to the (by now traditional) ITS data sequences derived from the molecularly very similar nuclear ribosomal region ETS. For the first time in this long history of phylogeny reconstruction, the resulting topology (Fig. 6B) placed *Dactylorhiza viridis* below the *incarnata* group (albeit with little statistical support in the case of the parsimony tree). The sequence of divergences in the remainder of the topology was largely familiar — next *D. aristata*, then the *D. romana* group, then the remainder of the genus — although these authors did also obtain a unique position for *D. foliosa* as sister to *D. fuchsii* (Druce) Soó plus *D. saccifera* (Brongn.) Soó. Crucially from the viewpoint of the present study, *D. iberica* was not included in their matrix.

Subsequent molecular phylogenetic analyses that were performed using the traditional 'candidate gene' approach (i.e. targeting particular genes for Sanger sequencing) have added little to the story (Bateman 2012a). When yet again exploring phylogeny across tribe Orchideae, Inda *et al.* (2010) effectively demonstrated the widely recognised chronic weakness of mitochondrial *coxI* data for reconstructing plant rather than animal phylogenies, but this did not dissuade them from later adding those mitochondrial data to the matrices developed by previous authors for nuclear ITS (Pridgeon *et al.* 1997; Bateman *et al.* 2003; Pillon *et al.* 2007) and plastid *rpl16* (Pillon *et al.* 2007). The three disparate datasets were for the first time analysed simultaneously, using Bayesian methods, thereby yielding a topology for *Dactylorhiza* that received strong statistical support (their Fig. 3). The sequence of divergence recovered by Inda *et al.* with increased bootstrap support was *incarnata* group > *viridis* > *iberica* > *romana* group > remainder, *D. iberica* yet again being represented by sample 960 (Fig. 6D).

By then it had become clear that the law of diminishing returns was affecting molecular phylogeny reconstruction in *Dactylorhiza*. Given the accumulated data, it had become justifiable to state that the three earliest-diverging lineages within the genus are the *D. incarnata* group, *D. iberica* and *D. viridis*, but we could not determine with any confidence which of the three taxa had the strongest claim on the coveted position of first-divergent. For us, this knowledge alone was more than sufficient to argue strongly that there was no legitimate case to be made for continuing to recognise *viridis* as a separate monotypic genus, '*Coeloglossum*' (Bateman 2009, 2012a).

What was clearly needed at this point was a further technological advance, and this was duly delivered from c. 2010 onwards in the form of several DNA-based techniques collectively termed next-generation sequencing (NGS: e.g. Olson *et al.* 2016). This category of techniques allows sequencing of vast numbers of DNA fragments; it is computationally challenging but yields several orders of magnitude more informative single-nucleotide polymorphisms (and thereby usually greater statistical robustness) than did candidate gene approaches. The initial NGS technique of choice for studies of Eurasian orchids has been RAD-seq (e.g. Davey *et al.* 2013; Olson *et al.* 2016), which has thus far been applied to *Ophrys* (Bateman *et al.* 2018), *Epipactis* (G. Sramkó *et al.* unpublished) and, happily, *Dactylorhiza* (M. Hedrén *et al.* unpublished). Details of the RAD-seq phylogeny of *Dactylorhiza* and *Gymnadenia* have yet to be published, but initial results show with

confidence that *D. iberica* and *D. viridis* constitute the two earliest-divergent lineages within the genus (admittedly, the relationship between these two species is presently less clearly resolved: M. Hedrén, M. Brandrud & O. Paun pers. comm. 2017).

The NGS-inspired hypothesis that *iberica* is a likely candidate for the role of earliest-divergent species in the genus *Dactylorhiza* should not come as a complete surprise. Most authors reviewing the taxonomy of the genus have in their formal classifications listed *D. iberica* either first (e.g. Vermeulen 1947, 1977; Soó 1960, 1962, 1980; Senghas 1968; Averyanov 1990) or last (Nelson 1976), implicitly acknowledging its morphological distinctness. They did so primarily on the grounds of raw morphological disparity, as an inevitable result of having employed phenetic (overall similarity) rather than cladistic (evolutionary relationship) concepts (e.g. Bateman 2001). More precisely, *D. iberica* was viewed as the most divergent of the dactylorchid species because it possesses several morphological characteristics unique within the genus, notably the commonly fusiform rather than digitate tubers, production of stolons, and incorporation of the lateral sepals into the hood that, in most other dactylorchids (and most of their closer relatives), is formed only by the median sepal plus lateral petals (Fig. 1A, B). The systematic outlier status of *D. iberica* has also been acknowledged more recently in certain allozyme (e.g. Hedrén 2001) and haplotype studies (reported in outline by Hedrén *et al.* 2007).

Finally, a morphological cladistic analysis pursued by Bateman *et al.* (in review), based on a matrix that has been informed by the above observations, yielded trees that effectively reconstructed the pre-molecular taxonomy of subtribe Orchidinae s.s. (one example is presented here as Fig. 6E). Intriguingly, *Dactylorhiza viridis* and *D. iberica* were shown (without bootstrap support) as sister-species. In all of the most-parsimonious trees this pairing diverged earliest within *Dactylorhiza*, although admittedly, in some trees the pair were pushed down the topology to an improbable position immediately below that of *Gymnadenia* s.l. (Fig. 6E).

It is difficult to obtain a genuinely objective overview of the wealth of information and broad spectrum of topologies summarised in Fig. 6, but it does seem reasonable to conclude that the earliest divergent lineage within the genus *Dactylorhiza* is either *D. iberica* or *D. viridis* (or perhaps both).

Indirect evidence of gene flow with other dactylorchids

Regrettably, both *Dactylorhiza viridis* and *D. iberica* have escaped involvement in published quantitative captive breeding programmes (e.g. Scopece *et al.* 2007). However, controlled crosses conducted by J. Haggar (pers. comm. 2007) showed that F_1 hybrids were readily produced between *D. viridis* and other, more derived dactylorchids, but that back-crosses were reliably wholly sterile. As an unusual side-benefit, the recognition by Pridgeon *et al.* (1997) that *D. viridis* was a *bona fide* member of the genus *Dactylorhiza* did enable horiculturalists to grow *D. viridis* more effectively (Hardwick 2000).

The number and frequency of natural hybrids of *Dactylorhiza viridis* has recently been summarised for the British Isles by Stace *et al.* (2015), although with the regrettable caveat that they elected to attribute *viridis* to *Coeloglossum* rather than *Dactylorhiza*. In the British Isles, *D. viridis* has been convincingly observed to hybridise with every other dactylorchid with which it comes into contact, excepting only the endemic tetraploids *D. traunsteinerioides* (Pugsley) R. M. Bateman & Denholm and *D. kerryensis* (Wilmott) P. F. Hunt & Summerh. It also hybridises naturally with several species of *Gymnadenia* s.l., sister genus to *Dactylorhiza* s.l., but not with the more distant outgroups *Pseudorchis* or *Platanthera*. Observations of such hybrids are infrequent and typically describe a single primary hybrid, thereby providing further (circumstantial) evidence that F_1 plants are sterile. Records of hybrids involving *D. viridis* are fewer in Continental Europe, although in a recent formal taxonomic treatment of such hybrids, Oddone *et al.* (2016) listed several combinations, including two involving species characteristic of Asia Minor.

We are not aware of any attempts having been made to involve *Dactylorhiza iberica* in artificial crossing experiments. However, Baumann (1983) listed hybrids reputedly observed between *D. iberica* and no less than six other dactylorchid species — in Greece with *D. saccifera* and *D. kalopissii* E. Nelson, and in Turkey with *D. incarnata*, *D. umbrosa* (Kar. & Kir.) Nevski, *D. nieschalkiorum* H. Baumann & Künkele and *D. urvilleana* (Steud.) H. Baumann & Künkele (also reported in Lebanon). Kreutz (1998) not only listed but also illustrated natural hybrids formed between *D. iberica* and every widely recognised *Dactylorhiza* species that occurs in Turkey other than *D. viridis* and members of the *D. romana* group. Further study is desirable to determine F_1 fertility, which would in turn determine whether *D. iberica* can participate in the formation of hybrid swarms.

In their comprehensive meta-analysis of European orchid pollination, Claessens & Kleynen (2011) were unable to list any observed pollinators or fruit-set figures for *Dactylorhiza iberica*. The frequency of hybridisation with more derived dactylorchid species suggests that bees are probably important pollinators of *D. iberica*. Several small beetles and small bees have been observed pollinating *D. viridis*, but the summary of fruit-set figures is bimodal. Most observers have recorded fruit-set values below 35%, consistent with allogamy in a food-deceptive species, whereas Claessens & Kleynen themselves made observations averaging 65% (n = 9), more typical of allogamy in a nectar-rewarding species. Both *D. viridis* and *D. iberica* flower in the later half of the phenological

period encompassed by the genus as a whole, at least partly reflecting their preferences for high altitudes (and, in the case of *D. viridis*, high latitudes).

In summary, current evidence suggests that both *Dactylorhiza iberica* and *D. viridis* are at least dominantly allogamous and, when given the opportunity, will hybridise with at least most, and possibly all, other species of *Dactylorhiza* irrespective of ploidy level. Occurrences of such hybrids are frequent but small-scale, supporting experimental evidence that F_1 plants are of very low fertility. We offer these observations as further evidence in support of continuing to include *D. viridis* (and indeed *D. iberica*) within the genus *Dactylorhiza*. However, we recognise that similarly sporadic and infertile natural hybrids occur equally frequently between many species of *Dactylorhiza* and members of its sister genus, *Gymnadenia* s.l. (including the former genus *Nigritella*).

Possible taxonomic variation within *Dactylorhiza iberica* s.l.: are the disjunct Greek populations conspecific with those from Asia Minor?

Nelson (1976: 106 – 107) argued that Greek and Turkish populations of *Dactylorhiza iberica* were similar in vegetative characteristics but differed in several floral characters, some of them admittedly being trends rather than reliable differences (Table 2). Consideration of the wide range of images available today, either published or placed on the Web, supports Nelson's contention, although all (rather than some) of the supposed distinguishing characters are probably best viewed as trends rather than reliable distinctions. Unsurprisingly, the available images also suggest that populations occurring on Cyprus resemble closely those found in Turkey. Specifically, plants from Greece (illustrated by Petrou *et al.* 2011; Antonopoulos 2015) tend to have comparatively lax inflorescences of flowers that are smaller, paler and less boldly marked, with narrower labella and lateral sepals bound more tightly into the hood, compared with those from Turkey (illustrated by Sundermann 1980; Baumann & Künkele 1982; Buttler 1991; Kreutz 1998) and Cyprus (illustrated by Kreutz 2004; Delforge 2006). Here, we have imaged a Greek plant in Fig. 1C but a Turkish plant in Figs 4 and 5, and the contrast in relative labellum dimensions at least is evident.

Many previous authors have emphasised the comparatively low (morphological) variation in, and paucity of hybrids involving, *Dactylorhiza iberica*. Unusually for a dactylorchid species, no infraspecific taxa are presently in common use by orchid enthusiasts. Yet two varieties of *D. iberica* were listed by Koch (1849) and four formas by Reichenbach (1851); each infraspecific taxon was said to be distinguished by only one or at most two features, most of them vegetative. Unfortunately, Reichenbach did not indicate the geographic locations of his infraspecific taxa. Moreover, such accusations of low phenotypic variability are not borne out by the few publications that illustrate several inflorescences (notably Kreutz 1998), nor are they supported by the few genetic studies that analysed more than one accession of *D. iberica* (Pillon *et al.* 2007; Hedrén *et al.*, unpublished). Lastly, we cannot yet eliminate the possibility that *D. iberica* shares the propensity of other species of *Dactylorhiza* (and of its sister genus, *Gymnadenia*) toward polyploidy, given that *D. iberica* was listed as unknown for karyotype in the *Flora Europaea* checklist (Moore 1982) and only one chromosome count has since been obtained (yielding the classic diploid number for the genus, $2n = 40$: Hedrén *et al.* 2007).

Obviously, these anecdotal observations of morphological variation require scientific exploration via morphometric field surveys (e.g. Bateman 2001, 2011), which are now nearing completion for *Dactylorhiza viridis* and underway for *D. iberica*. In addition, field observations of hybridisation and possible introgression could usefully be tested through controlled crossing (cf. Scopece *et al.* 2007).

Possible taxonomic variation within *Dactylorhiza viridis* s.l.: are ploidy differences evident?

The extensive circumboreal distribution of *Dactylorhiza viridis* might from first principles be predicted to offer ample opportunity for the intensity of taxonomic subdivision that has through the years afflicted most other species of the genus. In practice, variability within *D. viridis* has attracted more attention from authors in North America (e.g. Luer 1975; Sheviak & Catling 2002) than from those operating largely in Europe or Asia. Several infraspecific epithets have been suggested during the last two centuries, most of which rely primarily on differences in the vegetative robustness of the plants in general and the length of the bracts in particular. The more vigorous phenotypes reportedly dominate populations in North America and Japan, although they also occur elsewhere in the distribution of the species. These infraspecific taxa have most commonly been recognised at varietal rank, although occasionally they have been raised to the level of subspecies (Richter 1890; Hultén 1943; Kreutz 2007) or even species (Parlatore 1860). Thus far, only one infraspecific epithet has carried over from *'Coeloglossum viride'* or *'Habenaria viridis'* in order to be made under *Dactylorhiza*, viz *D. viridis* var. *virescens* (Muhl. ex Willd.) Baumbach (2013) (a phenotype that is arguably more appropriately awarded the epithet *'bracteata'*, which was established three pages before *'virescens'* in the treatment published by Muhlenberg in Willdenow 1805).

As noted by Sheviak & Catling (2002), populations of unusually vigorous plants occur sporadically across the range of *Dactylorhiza viridis*. Those occurring in England are on average significantly more robust and produce basal bracts 15 – 30 mm long. We agree with Sheviak & Catling that these features alone are an insubstantial basis for formal recognition, but the British populations also

Table 2. Morphological distinctions inferred by Nelson (1976) between populations of *Dactylorhiza iberica* in the mountains of Greece versus those of Turkey. Information tabulated by the present authors, who added asterisks to indicate those statements that in their opinion reflect broad trends rather than reliable distinctions. Note that the holotype was reputedly obtained by von Bieberstein (in Willdenow 1805) from the near the eastern end of the species' reported distribution, in present-day Georgia. Populations in Greece, Cyprus and Turkey are currently under comparison by the authors.

Character	Greece	Turkey (and Cyprus?)
Inflorescence	lax	denser*
Bracts	short	longer*
Hood	compact	more open*
Labellum shape	narrow	broader
Labellum markings	small	larger
Flower colour (pink)	pale	darker*

typically flower at least two weeks earlier than the nominate race and have provided circumstantial evidence of being tetraploid (presumably autotetraploid: R. Bateman & I. Denholm, unpublished), despite repeated assertions that *D. viridis* presents the standard dactylorchid chromosome compliment of $2n = 40$ (e.g. Moore 1982; Sheviak & Catling 2002). If our ongoing morphometric and flow cytometric studies confirm this inference, formal recognition would then be justified at a higher rank than varietas.

Further encouragement to update infraspecific taxa is provided by molecular studies. Pillon *et al.* (2007) reported divergence in the ITS region of up to seven steps (i.e. c. 1%, including substantial indels) between the small numbers of European and Chinese accessions available to them, and found a larger number of plastid haplotypes in *Dactylorhiza viridis* than have ben detected in any other dactylorchid. Stevens *et al.* (2010) analysed 38 European and four Chinese plants of *D. viridis* for plastid microsatellites. Despite employing only four polymorphic loci, they detected 14 haplotypes, four of which were unique to China and the more frequent of which spanned Europe. Admittedly, little obvious geographic structure emerged from the data, but this observation might be more readily explained if contrasting ploidy levels are indeed present within *D. viridis*. This outcome would not be entirely surprising, given that other, more derived species within the genus have become model systems for the study of both auto- and allopolyploidy (e.g. Hedrén *et al.* 2001; Pillon *et al.* 2007; Paun *et al.* 2010, 2011).

Lastly, we will briefly consider habitat preferences. Many *Dactylorhiza* species (including *D. iberica*) are wetland specialists, and most have requirements for at least a moderate amount of soil moisture during the growing period. However, *D. viridis* appears to include multiple ecotypes that specialise in contrasting soil moistures and pH values, ranging from mildly acidic moist moorlands to dry chalk downland. It is not therefore surprising that *D. viridis* shares mycorrhizal associates with *D. maculata* and *D. sphagnicola* Soó — two other dactylorchids capable of occupying moderately acid soils (Jacquemyn *et al.* 2016). Nonetheless, analogous situations occur elsewhere in the genus; for example, several contrasting (but almost genetically identical: Hedrén 2009) ecotypes of *D. incarnata* exhibit radically different pH preferences, and several ecotypes of *D. fuchsii* differ considerably in ranges of tolerance for both pH and soil moisture (Bateman & Denholm 1989).

Finally resolving two centuries of argument regarding optimal genus-level assignment of the Frog Orchid

We reproduce below an abstracted version of the self-imposed rules that we have consistently used to derive generic circumscriptions from an evolutionary tree, irrespective of whether that tree is based on molecular data, morphological data, or both. In aggregate, these criteria are sufficient to generate an explicit, logical, robust and biologically justifiable classification. Although each rule focuses on comparisons made *within* a single phylogenetic tree, note that the strongest tests of the reliability of particular branches (and thus of potential genus-level circumscriptions) are provided by comparisons *between* trees that are based on the same range of species but on contrasting categories of data. The rules are listed below in order of decreasing importance (cf. Bateman 2009: 253 – 254; Bateman 2012a: 102; Tang *et al.* 2015: 22 – 23):

Rule 1. Recognise only monophyletic groups (clades: evolutionarily inclusive, self-circumscribing groups) evident in the tree.
Rule 2. Preferentially divide the tree at branches that are comparatively statistically robust (usually also comparatively long); these are the branches that are most likely to survive further testing and hence yield the most stable classifications.
Rule 3. Minimise the proportion of branches in the tree that simultaneously represent more than one taxonomic rank (most notably, any terminal branch that represents not only a species but also a supposedly monotypic genus); by definition, recognising multiple ranks on a single branch cannot provide additional grouping information.
Rule 4. Preferentially divide the tree in a way that, within the existing Linnean system of formal nomenclature, minimises the need to (a) create new names and (b) create new combinations of existing names.

Applying the four rules listed above, Rule 1 (monophyly) precluded recognition of *Coeloglossum* as a separate genus in most of the published molecular trees (Fig. 6), the sole exception being that of Devos *et al.* (2006b). However, all molecular phylogenies, including

that of Devos *et al.* (2006b), fail Rule 2; the branch separating *Dactylorhiza viridis* from the other *Dactylorhiza* species is consistently no longer than the branches subtending *D. iberica* or the *D. incarnata* group, whereas the branch leading to the universally acknowledged sister genus of *Dactylorhiza*, viz. *Gymnadenia* s.l., is significantly longer than any branch within *Dactylorhiza* s.l.; this fact alone justifies the continued recognition of *Dactylorhiza* as genus separate from *Gymnadenia*. However, given that the most recent, data-rich studies emphasise that *D. iberica* may have diverged earlier than *D. viridis*, it would not be possible to recognise *D. viridis* as a separate genus without also recognising *D. iberica* as yet another separate genus. Adopting this radical approach would also contravene Rule 3; the supposed genus *'Coeloglossum'* and any novel genus based on *D. iberica* would each be monotypic and so neither supposed genus would, by definition, provide any grouping information. Lastly, Rule 4 has a negligible impact on this particular conundrum, given that we deliberately avoided major nomenclatural changes when eliminating the genus *Coeloglossum* by conserving the genus name *Dactylorhiza* against *Coeloglossum*, so that only one new nomenclatural combination was required (i.e. *C. viride* simply became *D. viridis*: Cribb & Chase 2001).

As previously noted by Bateman (2009, 2012a), the case of *'Coeloglossum'* illustrates well the fundamental difference between overall phenetic distance (given three taxa, which two are most similar?) and phylogeny reconstruction (given three taxa, which two are most closely related — in other words, which two have the most recent shared ancestor?). For most of the history of taxonomy, some form of similarity (typically assessed crudely, in the absence of either explicit rules or rigorous quantification) has been dominant, whereas today it is generally recognised that similarity measures are more appropriately subordinated to measures of closeness of relationship — certainly when circumscribing supraspecific taxa. We can see no good reason for making either *Dactylorhiza viridis* or *D. iberica* exceptions to this universal rule.

We would also argue that the surviving supporters of *'Coeloglossum'* have given inadequate consideration to the fact that clades tend to evolve in fractal fashion, most of the early innovation leading to greater phenotypic disparity than is achieved by later, less radical evolutionary tinkering with the products of the initial diversification event. We would predict from first principles that, given their role as early-divergent lineages within *Dactylorhiza*, *D. viridis* and *D. iberica* would possess comparatively more autapomorphic phenotypic character states (and would also share more plesiomorphic character states with genera closely related to *Dactylorhiza*). This prediction would be further enhanced if previous authors studying ontogenetic series of buds have been correct to ascribe the distinctive floral morphology of *D. viridis* to paedomorphosis, underpinned by pleiotropy that reflects small modifications of key genes that dictate floral development (Box *et al.* 2008).

Having said that, the belated inclusion of *Dactylorhiza iberica* in comparative studies clearly shows that the case for *D. viridis* being far more morphologically distinctive than any other *Dactylorhiza* species has been exaggerated by some observers; the two species are subtended by terminal branches of similar length in the morphological cladistic trees of Bateman *et al.* (in review). *Dactylorhiza iberica* possesses several characteristics that are unique or rare within the genus, both vegetative (e.g. fusiform or near-fusiform tubers, the formation of stolons, and production of unusually narrow leaves resembling those of *Gymnadenia*) and floral (e.g. the numerous glandular trichomes that adorn the adaxial surface of the labellum). Also, when flower morphology is considered, a few similarities become evident between *D. viridis* and *D. iberica*; these include incorporation of the lateral sepals into the hood protecting the column, possession of a labellum that is longer than wide and expands into a spur of only modest dimensions, and the absence of papillae within the spur; the tentative pairing of these two species shown in Fig. 6E therefore has some merit. Also, in the warm-temperate zone, both species occur only at fairly high altitudes (typically above 700 m asl) and flower later than most other dactylorchids: June – July (– August), depending on altitude and latitude.

In summary, there is no question in our minds that the most appropriate taxonomic decision, based on extensive and diverse scientific evidence, is to retain both *viridis* and *iberica* within *Dactylorhiza*.

Conclusions

(1) Recently generated NGS data support the assertion of Devos *et al.* (2006b) that *Dactylorhiza viridis* diverged earlier than the *D. incarnata* group, contradicting the tentative conclusion of most earlier ITS-only studies that *D. incarnata* diverged first (Fig. 6). However, the absence of *D. iberica* from the analysis performed by Devos *et al.* meant that their assertion that *D. viridis* diverged earlier than any other dactylorchid was relative rather than absolute, and is now further challenged by increased knowledge of *D. iberica* — a species long regarded as both distinct and primitive by plant morphologists. This outcome ably illustrates the importance of comprehensive taxon sampling previously emphasised by Bateman (2009, 2012a). It also cautions against Eurocentric selection of species for systematic comparison; answers to ongoing questions about the relationships among European orchid species often lie in regions of Asia, especially Asia Minor.

(2) The assertion of Devos *et al.* (2006b), Tyteca & Klein (2008) and of many other 21st Century authors — that *'Coeloglossum'* can legitimately remain a distinct monotypic genus as long as ambiguities remain in our understanding of its phylogenetic position — cannot be sustained. The most recent evidence weakens the previous claim of *Dactylorhiza incarnata* to be the earliest-diverging lineage

within *Dactylorhiza*, but the ongoing ambiguity in the placement of *D. viridis* relative to *D. iberica* is unlikely to be erased in the foreseeable future. Indeed, it was that very ambiguity that encouraged us to include *'Coeloglossum'* within *Dactylorhiz*a; we prefer to circumscribe genera on the basis of robust phylogenetic branches.

(3) If *Dactylorhiza viridis* and *D. iberica* did indeed diverge before the remaining species of *Dactylorhiza*, it has not escaped our attention that any 'stamp-collecting' taxonomist still determined to preserve '*Coeloglossum*' as a monotypic genus could do so simply by assigning *D. iberica* to a new monotypic genus, to be erected in parallel with *'Coeloglossum'*. Such an action would effectively by-pass the monophyly test, but would fail our three remaining classificatory rules — for confidence in monophyly, and avoiding monotypic genera, and minimising nomenclatural changes.

(4) *Dactylorhiza viridis* is no more morphologically divergent than is *D. iberica*, and neither species is strongly molecularly divergent. If the levels of divergence achieved by *'Coeloglossum'* were to be used as the yardstick for classifying all other Eurasian orchids, logical consistency would also require renewed separation of *Listera* from *Neottia*, *Nigritella* from *Gymnadenia*, *Comperia* and *Barlia* from *Himantoglossum*, *A. pyramidalis* from the remainder of *Anacamptis* s.l. (though perhaps not in one case — that of separating *Aceras anthropophora* from *Orchis*). Two decades of taxonomic progress would thus be erased in a single stroke (Bateman 2009, 2012a). The ongoing use of level of divergence as an argument for continued recognition of *'Coeloglossum'* without also indulging in the above regressive genus-level re-circumscriptions would therefore represent a clear case of special pleading.

(5) The lack of studies of variation in either the genetic or morphometric properties of *Dactylorhiza iberica* has left this pivotal species unusually poorly understood, and probably seriously *mis*understood. Neither karyotypes nor genome sizes have yet been explored, and Claessens & Kleynen (2011) were unable to list any pollination or fruit-set observations on the species in their comprehensive summary of European orchid pollinators (their Appendices 1 and 2). Nor are we aware of any previous attempt having been made to explore the micromorphology of *D. iberica*.

(6) Moreover, it has been decidedly unhelpful that the number of molecular phylogenetic studies that included *Dactylorhiza iberica* has exceeded the number of *D. iberica* plants from which DNA has been extracted! Improbable ITS and plastid sequences obtained first by Pridgeon *et al.* (1997) and subsequently perpetuated have compromised attempts to place what has proved to be a phylogenetically crucial species. Early molecular phylogenetic studies also (understandably) failed to explore the genetic variation present within *D. viridis*, which has since proved to be considerable.

(7) Nonetheless, even the modest amount of infraspecific data currently available to us suggests that sufficient variation, both morphological and molecular, is present within both *Dactylorhiza viridis* s.l. and *D. iberica* s.l. to warrant recognition of infraspecific taxa using criteria suggested by Bateman (2001, 2011, 2012b). Indeed, even multiple bona fide species may lurk within the entities currently named *D. viridis* and *D. iberica*. It will be essential to allow adequate science to be applied across each of the two groups *before* old epithets are exhumed for re-use or, worse still, new taxa are casually invented on the basis of little more than educated guesswork.

Acknowledgements

We thank Mikael Hedrén, Ovidiu Paun and Marie Brandrud for permission to very briefly describe relationships revealed during an ongoing RAD-seq study of *Dactylorhiza* s.l., and Ian Denholm plus an anonymous reviewer for providing constructive reviews of this manuscript.

References

Antonopoulos, Z. (2015). *Orchids of Greece (Hellas).* www.greekorchids.gr

Averyanov, L. V. (1990). A review of the genus *Dactylorhiza*. In: J. Arditti (ed.), *Orchid biology: reviews and perspectives*, V: 159 – 206. Timber Press, Portland.

Bateman, R. M. (2001). Evolution and classification of European orchids: insights from molecular and morphological characters. *J. Eur. Orch.* 33: 33 – 119.

____ (2009). Evolutionary classification of European orchids: the crucial importance of maximising explicit evidence and minimising authoritarian speculation. *J. Eur. Orch.* 41: 243 – 318.

____ (2011). The perils of addressing long-term challenges in a short-term world: making descriptive taxonomy predictive. In: T. R. Hodkinson, M. B. Jones, S. Waldren & J. A. N. Parnell (eds), *Climate change, ecology and systematics*. Systematics Association Special Volume, vol. 78: 67 – 95. Cambridge University Press, Cambridge.

____ (2012a). Circumscribing genera in the European orchid flora: a subjective critique of recent contributions. *Ber. Arbeitskr. Heimische Orchid.* 29: 92 – 124.

____ (2012b). Circumscribing species in the European orchid flora: multiple datasets interpreted in the context of speciation mechanisms. *Ber. Arbeitskr. Heimische Orchid.* 29: 160 – 212.

____ & Denholm, I. (1989). A reappraisal of the British and Irish dactylorchids, 3. The spotted-orchids. *Watsonia* 17: 319 – 349.

____ & Denholm, I. (2003). The Heath Spotted-orchid (*Dactylorhiza maculata* (L.) Soó) in the British Isles: a cautionary case-study in delimitating infraspecific taxa and inferring their evolutionary relationships. *J. Eur. Orch.* 35: 3 – 36.

____, Hollingsworth, P. M., Preston, J., Luo, Y.-B., Pridgeon, A. M. & Chase, M. W. (2003). Molecular phylogenetics and evolution of Orchidinae and selected Habenariinae (Orchidaceae). *Bot. J. Linn. Soc.* 142: 1 – 40.

____, Pridgeon, A. M. & Chase, M. W. (1997). Phylogenetics of subtribe Orchidinae (Orchidoideae, Orchidaceae) based on nuclear ITS sequences. 2. Infrageneric relationships and taxonomic revision to achieve monophyly of *Orchis sensu stricto*. *Lindleyana* 12: 113 – 141.

____, Sramkó, G. & Paun, O. (2018). Integrating restriction site-associated DNA sequencing (RAD-seq) with morphological cladistic analysis clarifies evolutionary relationships among major species groups of bee orchids. *Ann. Bot.* 121: c. 18.

Baumann, H. (1983). [Hybrid combinations involving *Dactylorhiza iberica*] *Ber. Arbeitskr. Heimische Orchid.* 15: incl. 74 – 80.

____ & Künkele, S. (1982). *Die wildwachsenden Orchideen Europas*. Kosmos, Stuttgart.

Baumbach, N. (2013). [New combinations in *Dactylorhiza*]. *Orchidee* 64: incl. 137.

Bell, A. K., Roberts, D. L., Hawkins, J. A., Rudall, P. J., Box, M. S. & Bateman, R. M. (2009). Comparative morphology of nectariferous and nectarless labellar spurs in selected clades of subtribe Orchidinae (Orchidaceae). *Bot. J. Linn. Soc.* 160: 369 – 387.

Box, M. S., Bateman, R. M., Glover, B. J. & Rudall, P. J. (2008). Floral ontogenetic evidence of repeated speciation via paedomorphosis in subtribe Orchidinae (Orchidaceae). *Bot. J. Linn. Soc.* 157: 429 – 454.

Buttler, K. P. (1991). *Field guide to orchids of Britain and Europe*. Crowood, Swindon.

Claessens, J. & Kleynen, J. (2011). *The flower of the European orchid: form and function*. Published by the authors, Voerendaal, Netherlands.

Cribb, P. J. & Chase, M. W. (2001). Conservation of *Dactylorhiza* (1481). Proposal to conserve the name *Dactylorhiza* Necker ex Nevski over *Coeloglossum* Hartm. (Orchidaceae). *Taxon* 50: 581 – 582.

Davey, J. W., Cezard, T., Fuentes-Utrilla, P., Eland, C., Gharbi, K. & Blaxter, M. L. (2013). Special features of RAD Sequencing data: implications for genotyping. *Molec. Ecol.* 22: 3151 – 3164.

Delforge, P. (2006). *Orchids of Europe, North Africa and the Middle East*. A. & C. Black, London.

Devos, N., Raspé, O., Jacquemart, A.-L. & Tyteca, D. (2006b). On the monophyly of *Dactylorhiza* Necker ex Nevski (Orchidaceae): is *Coeloglossum* (L.) Hartman a *Dactylorhiza*? *Bot. J. Linn. Soc.* 162: 261 – 269.

____, ____, Oh, S.-H., Tyteca, D. & Jacquemart, A.-L. (2006a). The evolution of *Dactylorhiza* (Orchidaceae) allotetraploid complex: insights from nrDNA sequences and cpDNA PCR-RFLP data. *Molec. Phylogenet. Evol.* 38: 767 – 778.

Gamarra, R., Galán, P., Pedersen, H. A., Ortúnez, E. & Sanz, E. (2015). Seed micromorphology in *Dactylorhiza* Necker ex Nevski (Orchidaceae) and allied genera. *Turk. J. Bot.* 39: 298 – 309.

Hardwick, C. (2000). The cultivation of *Coeloglossum viride*. *Hardy Orchid Soc. Newslett.* #15: 17.

Hedrén, M. (2001). Systematics of the *Dactylorhiza euxina/incarnata/maculata* polyploid complex (Orchidaceae) in Turkey: evidence from allozyme data. *Pl. Syst. Evol.* 229: 23 – 44.

____ (2009). Plastid DNA haplotype variation in *Dactylorhiza incarnata* (Orchidaceae): evidence for multiple independent colonization events into Scandinavia. *Nordic J. Bot.* 27: 69 – 80.

____, Fay, M. & Chase, M. W. (2001). Amplified fragment length polymorphisms (AFLP) reveal details of polyploid evolution in *Dactylorhiza* (Orchidaceae). *Amer. J. Bot.* 88: 1868 – 1880.

____, Nordström, S., Hovmalm, H. A. P., Pedersen, H. A. & Hansson, S. (2007). Patterns of polyploidy evolution in Greek marsh orchids (*Dactylorhiza*; Orchidaceae) as revealed by allozymes, AFLPs, and plastid DNA data. *Amer. J. Bot.* 94: 1205 – 1218.

____, Nordström, S. & Bateman, R. M. (2011). Plastid and nuclear DNA marker data support the recognition of four tetraploid marsh orchids (*Dactylorhiza majalis s.l.*, Orchidaceae) in Britain and Ireland. *Biol. J. Linn. Soc.* 104: 107 – 128 (+ 4 electronic appendices).

Hultén, E. (1943). *Flora of Alaska and the Yukon*, Vol. 3. *Acta Univ. Lund.* 2, pt. 39.

Inda, L. A., Pimental, M. & Chase, M. W. (2010). Contribution of mitochondrial *cox1* intron sequences to the phylogenetics of tribe Orchideae (Orchidaceae): do the distribution and sequence of this intron in orchids tell us something about its evolution? *Taxon* 59: 1053 – 1064.

____, ____ & ____ (2012). Phylogenetics of tribe Orchideae (Orchidaceae: Orchidoideae): based on combined DNA matrices: inferences regarding timing of diversification and evolution of pollination syndromes. *Ann. Bot.* 110: 71 – 90.

Jacquemyn, H., Waud, M., Merckx, V. S. F. T., Brys, R., Tyteca, D., Hedrén, M. & Lievens, B. (2016). Habitat-driven variation in mycorrhizal communities in the terrestrial orchid genus *Dactylorhiza*. *Nature. Sci. Rep.* 6: 37182. https://doi.org/10.1038/srep37182.

Koch, K. (1849). Beiträge zur einer Flora des Orients. *Linnaea* 22: [incl. 282].

Kreutz, C. A. J. (1998). *Die Orchideen der Türkei*. Published by the author, Landgraaf.

____ (2004). *The orchids of Cyprus*. Published by the author, Landgraaf.

____ (2007). New combinations and additions regarding various European orchid taxonomies. *Ber. Arbeitskr. Heimische Orchid.* 24: 142 – 186.

Luer, C. (1975). *The native orchids of the United States and Canada, excluding Florida*. New York Botanical Garden, New York.

Moore, D. M. (1982). *Flora Europaea check-list and chromosome index*. Cambridge University Press, Cambridge.

Nelson, E. (1976). *Monographie und Ikonographie der Orchiaceen-Gattung, III*. Dactylorhiza. Published by the author, Zürich.

Oddone, N., Andreoli, M. V. & Casabianca, A. (2016). New genus subdivisions and taxonomic revision of intergeneric hybrids in genera *Dactylorhiza*, *Gymnadenia*, *Pseudorchis* and in nothogenera ×*Pseudorhiza*, ×*Pseudadenia* and ×*Dactylodenia* (*Orchidaceae*). *GIROS Orch. Spont. Eur.* 59: 347 – 377.

Olson, P. D., Hughes, J. & Cotton, J. A. (eds) (2016). *Next generation systematics*. Systematics Association Special Volume, vol. 85. Cambridge University Press, Cambridge.

Parlatore, F. (1860). *Flora Italiana 3*. Le Monnier, Florence.

Paun, O., Bateman, R. M., Fay, M. F., Hedrén, M., Civeyrel, L. & Chase, M. W. (2010). Stable epigenetic effects impact evolution and adaptation in allopolyploid orchids. *Molec. Biol. Evol.* 27: 2465 – 2473.

____, ____, Luna, J. A., Moat, J., Fay, M. F. & Chase, M. W. (2011). Gene expression alteration and ecological divergence in sibling allopolyploids of *Dactylorhiza* (Orchidaceae). *BMC Evol. Biol.* 11: 113 (14 pp.).

Petrou, N., Petrou, M. & Giannakoulias, M. (2011). *Orchids of Greece*. Koan, Athens.

Pillon, Y., Fay, M. F., Hedrén, M., Bateman, R. M., Devey, D., Shipunov, A., van der Bank, M. & Chase, M. W. (2007). Insights into the evolution and biogeography of Western European species complexes in *Dactylorhiza* (Orchidaceae). *Taxon* 56: 1185 – 1208.

____, ____, Shipunov, A. B. & Chase, M. W. (2006). Species diversity versus phylogenetic diversity: a practical study in the taxonomically difficult genus *Dactylorhiza* (Orchidaceae). *Biol. Conserv.* 129: 4 – 13.

Pridgeon, A. M., Bateman, R. M., Cox, A. V., Hapeman, J. R. & Chase, M. W. (1997). Phylogenetics of subtribe Orchidinae (Orchidoideae, Orchidaceae) based on nuclear ITS sequences. 1. Intergeneric relationships and polyphyly of *Orchis sensu lato*. *Lindleyana* 12: 89 – 109.

Reichenbach, H. G. (1851). Orchideae. *Icones Florae Germanicae et Helveticae*, 13/14. Leipzig.

Richter, K. (1890). *Plantae Europae: enumeratio systematica et synonymica plantarum phaenerogamicarum in Europa sponte crescentium vel mere inquilinarum 1*. Engelmann, Leipzig.

Scopece, G., Cozzolino, S., Musacchio, A. & Widmer, A. (2007). Patterns of reproductive isolation in Mediterranean deceptive orchids. *Evolution* 61: 2623 – 2642.

Senghas, K. (1968). Taxonomische Ubersicht der Gattung *Dactylorhiza* Necker ex Nevski. *Jahresber. Naturwiss. Vereins Wuppertal* 21 – 2: 32 – 67.

Sheviak, C. J. & Catling, P. M. (2002). *Coeloglossum* Hartman. In: *Flora of North America Editorial Committee (eds), Flora of North America North of Mexico* 26: 580. Oxford University Press, Oxford.

Shipunov, A. B., Fay, M. F., Pillon, Y., Bateman, R. M. & Chase, M. W. (2004). *Dactylorhiza* (Orchidaceae) in European Russia: combined molecular and morphological analysis. *Amer. J. Bot.* 91: 1419 – 1426.

Soó, R. de. (1960). Synopsis generis *Dactylorhiza* (*Dactylorchis*). *Ann. Univ. Sci. Budapest. Rolando Eötvos. Sect. Biol.* 3: 335 – 357.

____ (1962). *Nomina Nova Generis Dactylorhiza*. Published by the author, Budapest.

____ (1980). *Dactylorhiza* Necker ex Nevski. In: T. G. Tutin, V. H. Heywood, N. A. Burgess, D. M. Moore, D. H. Valentine, S. M. Walters & D. A. Webb (eds), *Flora Europaea* 5: 333 – 337. Cambridge University Press, Cambridge.

Sramkó, G., Molnár, A. V., Hawkins, J. A. & Bateman, R. M. (2014). Molecular phylogenetics and evolution of the Eurasiatic orchid genus *Himantoglossum s.l. Ann. Bot.* 114: 1609 – 1626.

Stace, C. A., Preston, C. D. & Pearman, D. A. (2015). *Hybrid flora of the British Isles*. BSBI, Bristol.

Stevens, L. J., Campbell, A. J., Cowan, R. S. & Fay. M. J. (2010). Preliminary study to detect genetic variation within *Dactylorhiza viridis* using plastid microsatellites. Unpublished report to English Nature, 8 pp. Royal Botanic Gardens Kew.

Sundermann, H. (1980). *Europäische und mediterrane Orchideen: eine Bestimmungsflora*, 3rd ed. Schmersow, Hildesheim.

Tang, Y., Yukawa, T., Bateman, R. M., Jiang, H. & Peng, H. (2015). Phylogeny and classification the East Asian *Amitostigma* alliance (Orchidaceae: Orchideae) based on six DNA markers. *BioMed Centr. Evol. Biol.* 15: e96 [32 pp.] [doi: https://doi.org/10.1186/s12862-015-0376-3]

Tyteca, D. & Klein, E. (2008). Genes, morphology and biology — the systematics of *Orchidinae* revisited. *J. Eur. Orch.* 40: 501 – 544.

____ & ____ (2010). Genes, morphology and biology — the systematics of Orchidinae revisited: a reappraisal. *J. Eur. Orch.* 41: 473 – 480.

Vermeulen, P. (1947). *Studies on dactylorchids*. Schotanus & Jens, Utrecht.

____ (1977). Orchideeën, systematisch ingedeeld. In: J. Landwehr (ed.), *Wilde orchideeën van Europa*, pp. 551 – 557. VBNN, Amsterdam.

Willdenow, C. L. (1805). *Species Plantarum 4*. Published by the author, Berlin.