



Molecular phylogenetics of *Vanda* and related genera (Orchidaceae)

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The genus *Vanda* and its affiliated taxa are a diverse group of horticulturally important species of orchids occurring mainly in South-East Asia, for which generic limits are poorly defined. Here, we present a molecular study using sequence data from three plastid DNA regions. It is shown that *Vanda s.l.* forms a clade containing approximately 73 species, including the previously accepted genera *Ascocentrum*, *Euanthe*, *Christensonia*, *Neofinetia* and *Trudelia*, and the species *Aerides flabellata*. Resolution of the phylogenetic relationships of species in *Vanda s.l.* is relatively poor, but existing morphological classifications for *Vanda* are incongruent with the results produced. Some novel species relationships are revealed, and a new morphological sectional classification is proposed based on support for these groupings and corresponding morphological characters shared by taxa and their geographical distributions. The putative occurrence of multiple pollination syndromes in this group of taxa, combined with complex biogeographical history of the South-East Asian region, is discussed in the context of these results. © 2013 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2013, 173, 549–572.

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INTRODUCTION

Vanda R.Br. comprises some 73 species (Gardiner & Cribb, 2013) and is widely distributed mainly throughout South-East Asia, from India and Nepal, through southern China to Korea and Japan, and southwards through the Philippines and Indonesia to northern Australia and the Solomon Islands (Govaerts, 2012). Many species are narrowly endemic, with a major centre of diversity in the South-East Asian archipelagos. *Vanda* spp. are epiphytes and

lithophytes with monopodial growth, stiffly erect and praemorse tipped leaves. Plant range from 20 cm to 2 m in height, with often brightly coloured, sometimes fragrant, flowers. There is great diversity in floral shape, colour and particularly labellum structure, and the genus is one of the five most horticulturally important orchid genera in the world. A number of species are threatened with extinction in the wild, being rare and/or geographically restricted in distribution (Motes, 1997).

The first species recorded in Western literature were documented by Rumphius in the late 17th century in his posthumously published magnum opus

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Herbarium Amboinensis (Rumphius, 1741). Linnaeus described two species of *Vanda* (as species of *Epidendrum* L.) in the first and second editions of *Species Plantarum* (Linnaeus, 1753, 1762). The genus *Vanda* was first established in 1795 by William Jones, who based his concept on *Epidendrum tessellatum* Roxb. (now known as *V. tessellata* (Roxb.) Hook. ex G. Don in J.C. Loudon), but the genus was not validly published until 1820 by Robert Brown (1820). By 1853, the genus comprised some 25 species, enough that John Lindley (1853) could re-circumscribe the genus, broadly with the same generic concept as Jones, but postulating five sections at the subgeneric level. Two of Lindley's sections have since been removed from *Vanda* and erected as one or two genera in their own right: section *Fieldia* Lindl. to *Vandopsis* Pfitzer in H.G.A. Engler & K.A.E. Prantl and *Dimorphorchis* Rolfe; and section *Anota* Lindl. to *Rhynchostylis* Blume.

Since Lindley's landmark work, the basic concept of *Vanda* has remained broadly constant until today, with additional species being described in the genus throughout the 20th century and into the 21st century. Only in the last 25 years have questions about the generic limits of *Vanda* troubled the image of a previously seemingly taxonomically stable genus. Motes (1997) described the genus *Vanda* as a taxonomic 'black pit' and Christenson (1987b) stated that it required 'complete taxonomic revision'. The placement of a number of small genera such as *Ascocentrum* Schltr., *Euanthe* Schltr., *Trudelia* Garay and *Christensonia* Haager in relation to *Vanda* has been debated and has until now remained uncertain. Clarification of the generic status of *Vanda* and a number of the taxa in the genus and a more systematic classification of *Vanda* spp. have long been required. The presence of praemorse leaf tips and a three-lobed labellum are good characters for *Vanda* (Seidenfaden, 1988), but the diversity in floral morphology seen across the species is extremely high, with a wide range of sizes, shapes, colours and labellum structures (including presence or absence of a spur, column foot and mid-lobe ornamentation).

Relationships in the wider subtribe Aeridinae are similarly confused. Characterized by monopodial growth, members of the subtribe all have a highly developed velamen on the roots, an entire rostellum, a relatively small spur formed by the lip and pollinaria with well-developed stipes and viscidia, two to four pollinia and, in several genera, a prominent column foot (Chase *et al.*, 2003; Topik, Yukawa & Ito, 2005). Attempts to clarify some of the relationships between genera of Aeridinae have been made most notably by Christenson (1985, 1986, 1987a, b, 1992, 1994) with a more 'classical' approach using floral and, to a lesser degree, non-reproductive characters.

In contrast, Senghas (1988a) and Seidenfaden (1988) based their classifications on pollinaria and column foot characters when publishing new species, combinations and genera and writing floristic accounts. Christenson (1994) wrote, concerning the great range of morphologies exhibited by the subtribe, that there was still much research needed to understand the species and generic limits of the group. In the only subtribe-wide molecular systematic study to date, based on plastid *matK* and nuclear ribosomal internal transcribed spacer (ITS) data, Topik *et al.* (2005) commented on the likelihood that past morphological diversification and potential parallelism in vegetative and reproductive characters have been significant contributors to the present difficulty of resolving relationships in the group. In their study, they included only *Vanda coerulea* Griff. ex Lindl., a species that is indisputably part of *Vanda*, but found a close relationship with *Aerides* Lour., *Christensonia*, *Trudelia* and *Neofinetia* Hu. Several recent molecular phylogenetic studies have been published on groups of species/genera in Aeridinae: *Aerides* (Kocyan *et al.*, 2008), *Holcoglossum* Schltr. (Fan *et al.*, 2009), leafless Vandaeae (Carlsward *et al.*, 2006) and *Phalaenopsis* Blume (Padolina, Linder & Simpson, 2005; Tsai, Huang & Chou, 2006, 2010), but none has to date concentrated specifically on *Vanda*.

The placement of a number of species and genera in relation to *Vanda* has remained a matter of debate, with authors disagreeing on their correct classification. *Ascocentrum* contains short-stemmed species, often with brightly coloured flowers (reds, oranges, purples) with approximately equal-sized sepals and petals, a long cylindrical nectar-filled spur, small side lobes and a ligulate epichile (strap-shaped apical portion of the labellum mid-lobe). None of the species has ever been placed in *Vanda*, although they hybridize readily in cultivation with *Vanda* spp., forming fertile hybrids (Christenson, 1987a; Motes, 1997; Topik *et al.*, 2005).

Kamemoto & Shindo (1962) studied the chromosomal relationships among many of the taxa in the *Vanda*–*Aerides* alliance (*sensu* Christenson 1987a), which includes *Aerides*, *Ascocentrum*, *Holcoglossum*, *Papilionanthe* Schltr., *Seidenfadenia* Garay and *Vanda*, and closely related genera such as *Euanthe*, *Neofinetia*, *Phalaenopsis*, *Renanthera* Lour., *Trichoglottis* Blume and *Arachnis* Blume. This work found little support for the genus *Euanthe* as distinct from *Vanda*, because of the perfect chromosomal pairing of *Euanthe sanderiana* (Rchb.f.) Schltr. (= *Vanda sanderiana* (Rchb.f.) Rchb.f.) with *Vanda* spp. and hybrids, and the resulting hybrids exhibit high fertility. Holtum (1958), in contrast, considered *Euanthe* as morphologically distinct from *Vanda*, with its distinctive lip shape, broad flat petals and sepals and large

lateral sepals. He, however, recognized the ease with which monotypic *Euanthe* hybridizes with *Vanda* spp. and stated that it 'may be a practical step to reunite it to *Vanda*'. The karyotypes of the strap-leaved *Vanda* spp. (at the time, *Holcoglossum* and *Papilionanthe* were still considered to be part of *Vanda*, as the terete-leaved species) and *Ascocentrum* have been shown to be extremely similar (Kamemoto & Shindo, 1962), with chromosomes similar in size and morphology pairing well during meiosis, as were those of *Neofinetia falcata* (Thunb.) Hu (= *Vanda falcata* (Thunb.) Beer). The chromosomes of the terete-leaved *Vanda* species do not pair well with those of the strap-leaved species, lending support to the exclusion of these taxa from the genus. A single random amplified polymorphic DNA (RAPD) study of a few vandaceous species has shown the single *Ascocentrum* species included and *Euanthe sandariana* nesting within a clade of strap-leaved *Vanda* spp., with the terete-leaved *Vanda teres* (Roxb.) Lindl. (= *Papilionanthe teres* (Roxb.) Schltr.) and *Vanda hookeriana* Rchb.f. (= *Papilionanthe hookeriana* (Rchb.f.) Schltr.) separated from this clade (Lim *et al.*, 1999).

Aerides flabellata Rolfe ex Downie (= *Vanda flabellata* (Rolfe ex Downie) Christenson) has been a focus of much taxonomic disagreement, and it was placed in *Aerides* by some (Seidenfaden, 1988, 1992) on account of characters such as a long column foot and motile lip, and *Vanda* by others because of the short spur and broad lip (Garay, 1972; Seidenfaden, 1973; Christenson, 1985, 1986; Motes, 1997; Kocyan *et al.*, 2008). Christenson (1994) treated the species as *nomina incertae sedis* until further study could be made. The more recently described species, *Christensonia vietnamica* Haager (= *Vanda vietnamica* (Haager) L.M.Gardiner), with morphological similarities to *Vanda* and *Rhynchostylis* (Christenson, 1994), has been affiliated with *A. flabellata*, being described as 'almost a yellow *A. flabellata*' (D. L. Roberts, pers. comm.). Recent molecular studies including *V. flabellata* and *V. vietnamica* have supported their placement in *Vanda* (Topik *et al.*, 2005; Carlswald *et al.*, 2006; Kocyan *et al.*, 2008; Fan *et al.*, 2009).

In summary, relationships in and around *Vanda* remain unclear, in spite of the recent molecular studies of other groups in Aeridinae. As Christenson (1987a) stated, the genus is a 'poorly known, imprecisely defined group held together by symplesiomorphies. *Vanda* requires a complete taxonomic revision'.

The main questions that this molecular study aimed to address were: (1) Is *Vanda*, as currently circumscribed, monophyletic, and what are the phylogenetic relationships of *Vanda* with related genera in Aeridinae? (2) Which morphology-based sectional classifications of *Vanda* are supported by molecular data? We sequenced three plastid DNA regions from

35 *Vanda* spp. and 22 species from other genera of Aeridinae to answer these questions.

MATERIAL AND METHODS

TAXON SAMPLING

One hundred and one individuals were sampled (Table 1), representing 35 species accepted as being placed in *Vanda* at the time of this study and 22 species from 14 Aeridinae genera (*Aerides*, *Ascocentrum*, *Christensonia*, *Dimorphorchis*, *Holcoglossum*, *Neofinetia*, *Papilionanthe*, *Phalaenopsis*, *Renanthera*, *Rhynchostylis*, *Sedirea* Garay & H.R.Sweet, *Seidenfadenia*, *Taprobanea* Christenson, *Vandopsis*). Taxon names and authorities used here are based on the World Checklist of Monocotyledons, with some modification (Govaerts, 2012), before recent nomenclatural changes were published by the first author of this paper (Gardiner, 2012). Because of difficulties involved in collecting wild material, specimens were obtained from cultivated species material from botanic gardens and specialist growers of vandaceous species. Multiple accessions of some species were included, where possible. Based on previous molecular studies of Aeridinae, *Phalaenopsis* (represented by *P. cornu-cervi* (Breda) Blume & Rchb.f.) was chosen as the outgroup for the rest of Aeridinae (Topik *et al.*, 2005; Carlswald *et al.*, 2006; Kocyan *et al.*, 2008; Fan *et al.*, 2009).

With a genus as horticulturally important as *Vanda*, the potential for accidental hybridization and introgression between cultivated specimens is high, especially if the morphological differences between species are not well understood. A number of additional specimens originally included in this study from cultivated sources were rejected from the final analyses on the basis of dubious genetic origin. After sequencing and incorporation into preliminary phylogenetic trees, several specimens sampled were shown to be of probable hybrid origin, and these sequences were removed from the analyses.

AMPLIFICATION AND SEQUENCING

DNA was extracted from flower or leaf material dried in silica gel, according to the guidelines of Chase & Hills (1991), using either one of two methods, the modified cetyl trimethylammonium bromide (CTAB)-chloroform protocol of Doyle & Doyle (1987) and the DNeasy Plant mini kit (Qiagen), following the manufacturer's recommended protocol.

Three plastid DNA regions were amplified using the primers listed in Table 2. The primers 19F (Gravendeel *et al.*, 2001), trnK-2R (Johnson & Soltis, 1994) and OMAT396F (Topik *et al.*, 2005) were used to amplify a region (referred to here as '*matK*')

Table 1. List of species names, geographic range, voucher specimens, and NCBI accession numbers for taxa used in this study

Species	Geographic range	Additional reference number in phylogeny	Voucher/herbarium	<i>matK</i>	<i>psbA</i>	<i>trnL-F</i>
<i>Vanda ampullacea</i> (Roxb.) L.M.Gardiner, in this study: <i>Ascocentrum ampullaceum</i>	China, Assam, Bangladesh, Nepal, Himalayas, Andaman Islands, Laos, Burma, Thailand, Vietnam		Gardiner, 23 (FLAS)	KC823030	KC985327	KC985416
<i>Vanda arcuata</i> J.J.Sm.	Sulawesi		0–5406 (SBG)	KC822954	KC985251	KC985340
<i>Vanda bensonii</i> Batem.	Assam, Burma, Thailand	1	Royal Botanic Gardens, Kew 12/2004-4 (K)	KC822955	KC985252	KC985341
<i>Vanda bensonii</i> Batem.		2	Swansea Botanical Complex, S19920413	KC822956	KC985253	KC985342
<i>Vanda brunnea</i> Rchb.f.	China, Burma, Thailand, Vietnam		Luxembourg Botanic Garden, LUX2001082	KC822957	KC985254	KC985343
<i>Vanda celebica</i> Rolfe	Sulawesi		Gardiner, 131 (K)	KC822958	KC985255	KC985344
<i>Vanda christensoniana</i> (Haager) L.M.Gardiner, in this study: <i>Ascocentrum christensoniana</i>	Vietnam		Chase, 15795 (K)	KC823031	KC985328	KC985417
<i>Vanda coerulea</i> Griff. ex. Lindl.	China, Assam, East Himalayas,	1	Gardiner, 121 (K)	KC822959	KC985256	KC985345
<i>Vanda coerulea</i> Griff. ex. Lindl.	Burma, Thailand	2	Chase 22871 (K)	KC822960	KC985257	KC985346
<i>Vanda coerulea</i> Griff. ex. Lindl.		3	Luxembourg Botanic Garden, LUX2002050	KC822961	KC985258	KC985347
<i>Vanda coerulescens</i> Griff.	China, Assam, East Himalayas,	1	Gardiner, 120 (K)	KC822962	KC985259	KC985348
<i>Vanda coerulescens</i> Griff.	Burma, Thailand	2	OR-303–1999 (SLZ)	KC822963	KC985260	KC985349
<i>Vanda coerulescens</i> Griff.		3	Lyon Botanic Garden, 900488	KC822964	KC985261	KC985350
<i>Vanda cristata</i> Wall. ex. Lindl.	China, Assam, Bangladesh,	1	Roberts, D.L., s.n. (K)	KC822965	KC985262	KC985351
<i>Vanda cristata</i> Wall. ex. Lindl.	India, Nepal, Burma, Vietnam	2	Lyon Botanic Garden, 890708	KC822966	KC985263	KC985352

Table 1. Continued

Species	Geographic range	Additional reference number in phylogeny	Voucher/herbarium	<i>matK</i>	<i>psbA</i>	<i>trnL-F</i>
<i>Vanda curvifolia</i> (Lindl.) L.M.Gardiner, in this study: <i>Ascocentrum curvifolium</i>	Laos, Burma, Thailand, Vietnam	1	Gardiner, 20 (FLAS)	KC823032	KC985329	KC985418
<i>Vanda curvifolia</i> (Lindl.) L.M.Gardiner, in this study: <i>Ascocentrum curvifolium</i>		2	Basel Botanic Garden, 1446/99WP (Z)	EF655789	KC693488	EF670423
<i>Vanda curvifolia</i> (Lindl.) L.M.Gardiner, in this study: <i>Ascocentrum curvifolium</i>		3	Gardiner, 16 (FLAS)	KC823033	KC985330	KC985419
<i>Vanda dearei</i> Rchb.f.	Borneo, Lesser Sunda Islands		Gardiner, 112 (K)	KC822967	KC985264	KC985353
<i>Vanda denisoniana</i> Benson & Rchb.f.	Laos, Burma, Thailand, Vietnam	1	Gardiner, 10 (FLAS) (K)	KC822968	KC985265	KC985354
<i>Vanda denisoniana</i> Benson & Rchb.f.		2	Gardiner, 25 (FLAS) (K)	KC822969	KC985266	KC985355
<i>Vanda denisoniana</i> Benson & Rchb.f.		3	Gardiner, 27 (FLAS) (K)	KC822970	KC985267	KC985356
<i>Vanda denisoniana</i> Benson & Rchb.f.		4	Lyon Botanic Garden, 980277	KC822971	KC985268	KC985357
<i>Vanda denisoniana</i> Benson & Rchb.f.		5	Luxembourg Botanic Garden, 1995-01	KC822972	KC985269	KC985358
<i>Vanda devoogtii</i> J.J.Sm.	Sulawesi		Gardiner, 135 (K)	KC822973	KC985270	KC985359
<i>Vanda falcata</i> (Thunb.) L.M.Gardiner, in this study: <i>Neofinetia falcata</i>	China, Japan, Korea		AK010824/1/01 (Z)	EF655782	KC693492	EF67021

Table 1. *Continued*

Species	Geographic range	Additional reference number in phylogeny	Voucher/herbarium	<i>matK</i>	<i>psbA</i>	<i>trnL-F</i>
<i>Vanda flabellata</i> (Rolfe ex Downie) Christenson	Yunnan, Laos, Myanmar, Thailand	1	BG Basel 1039/97 (Z)	EF655786	KC693485	EF670410
<i>Vanda flabellata</i> (Rolfe ex Downie) Christenson		2	Royal Botanic Gardens, Kew 12/04 (K)	KC822974	KC985271	KC985360
<i>Vanda flabellata</i> (Rolfe ex Downie) Christenson		3	Roberts, 2004-739 (K)	KC822975	KC985272	KC985361
<i>Vanda flavobrunnea</i> Rchb.f.	China, Assam, Nepal, Himalayas, Lao, Burma, Thailand, Vietnam, Sumatra		s.n. (Z)	EF655802	KC693486	EF670416
<i>V. furva</i> (L.) Lindl.	Moluccas		Gardiner, 204 (K)	KC822976	KC985273	KC985362
<i>Vanda hastifera</i> Rchb.f.	Borneo		Gardiner, 101 (K)	KC822978	KC985274	KC985363
<i>Vanda helvola</i> Blume	Borneo, Java, Malaysia,	1	Gardiner, 145 (K)	KC822979	KC985275	KC985364
<i>Vanda helvola</i> Blume	Sumatra, New Guinea	2	Gardiner, 206 (K)	KC822980	KC985276	KC985365
<i>Vanda helvola</i> Blume		3	Gardiner, 116 (K)	KC822981	KC985277	KC985366
<i>Vanda hindsii</i> Lindl.	New Guinea, Solomon	1	Gardiner, 103 (K)	KC822982	KC985278	KC985367
<i>Vanda hindsii</i> Lindl.	Islands, Queensland	2	Gardiner, 53 (FLAS) (K)	KC822983	KC985279	KC985368
<i>Vanda hindsii</i> Lindl.		3	Gardiner, 62 (FLAS) (K)	KC822984	KC985280	KC985369
<i>Vanda hindsii</i> Lindl.		4	Hortus Botanicus Leiden, 20030985 (L)	KC822985	KC985281	KC985370
<i>Vanda insignis</i> Blume	Lesser Sunda Islands		Gardiner, 180 (K)	KC822977	KC985282	KC985371
<i>Vanda jennae</i> O'Bryne & Verm.	Sulawesi		0-5665 (SBG) (K)	KC822986	KC985283	KC985372
<i>Vanda lamellata</i> Lindl.	Japan, Taiwan, Borneo, Philippines, Marianas		Gardiner, 59 (FLAS) (K)	KC822987	KC985284	KC985373
<i>Vanda lamellata</i> var. <i>boxallii</i> Rchb.f.	Philippines		Gardiner, 44 (FLAS) (K)	KC822988	KC985285	KC985374

Table 1. Continued

Species	Geographic range	Additional reference number in phylogeny	Voucher/herbarium	<i>matK</i>	<i>psbA</i>	<i>trnL-F</i>
<i>Vanda lamellata</i> var. <i>remediosae</i> Ames & Quisumb.	Philippines	1	Gardiner, 6 (FLAS) (K)	KC822989	KC985286	KC985375
<i>Vanda lamellata</i> var. <i>remediosae</i> Ames & Quisumb.		2	Gardiner, 18 (FLAS) (K)	KC822990	KC985287	KC985376
<i>Vanda lilacina</i> Teijsm. & Binn.	China, Cambodia, Laos, Burma,	1	Gardiner, 60 (FLAS) (K)	KC822991	KC985288	KC985377
<i>Vanda lilacina</i> Teijsm. & Binn.	Thailand, Vietnam	2	Lyon Botanic Garden, 900516	KC822992	KC985289	KC985378
<i>Vanda limbata</i> Blume	Java, Lesser Sunda Islands?	1	Gardiner, 161 (K)	KC822993	KC985290	KC985379
<i>Vanda limbata</i> Blume		2	Gardiner, 149 (K)	KC822994	KC985291	KC985380
<i>Vanda limbata</i> Blume		3	Gardiner, 61 (K)	KC822995	KC985292	KC985381
<i>Vanda lindenii</i> Blume	New Guinea	1	Gardiner, 240 (K)	KC822996	KC985293	KC985382
<i>Vanda lindenii</i> Blume		2	Gardiner, 110 (K)	KC822997	KC985294	KC985383
<i>Vanda liouvillei</i> Finet.	Assam, Laos, Burma,	1	Gardiner, 30 (FLAS) (K)	KC822998	KC985295	KC985384
<i>Vanda liouvillei</i> Finet.	Thailand, Vietnam	2	Gardiner, 31 (FLAS) (K)	KC822999	KC985296	KC985385
<i>Vanda luzonica</i> Loher ex Rolfe	Philippines	1	Gardiner, 107 (K)	KC823000	KC985297	KC985386
<i>Vanda luzonica</i> Loher ex Rolfe		2	Gardiner, 9 (FLAS) (K)	KC823001	KC985298	KC985387
<i>Vanda merrillii</i> Ames & Quisumb.	Philippines	1	Gardiner, 126a (K)	KC823002	KC985299	KC985388
<i>Vanda merrillii</i> Ames & Quisumb.		2	Lyon Botanic Garden, 012660	KC823003	KC985300	KC985389
<i>Vanda metusalae</i> O'Byrne	Sulawesi		0-4647 (SBG)	KC823004	KC985301	KC985390
<i>Vanda parviflora</i> Lindl.	Assam, India, Nepal, Sri Lanka, Himalayas, Burma, Thailand		Gardiner, 24 (FLAS) (K)	KC823005	KC985302	KC985391
<i>Vanda perplexa</i> Motes & D.L.Roberts	Lesser Sunda Islands		Gardiner, 12 (FLAS)	KC823006	KC985303	KC985392
<i>Vanda roeblingiana</i> Rolfe	Philippines		Gardiner, 124 (K)	KC823007	KC985304	KC985393

Table 1. *Continued*

Species	Geographic range	Additional reference number in phylogeny	Voucher/herbarium	<i>matK</i>	<i>psbA</i>	<i>trnL-F</i>
<i>Vanda sanderiana</i> (Rchb.f.) Rchb.f.	Philippines	1	Gardiner, 123 (K)	KC823008	KC985305	KC985394
<i>Vanda sanderiana</i> (Rchb.f.) Rchb.f.		2	Gardiner, 115 (K)	KC823009	KC985306	KC985395
<i>Vanda sanderiana</i> (Rchb.f.) Rchb.f.		3	Royal Botanic Gardens, Kew 12/04 (K)	KC823010	KC985307	KC985396
<i>Vanda sanderiana</i> (Rchb.f.) Rchb.f.		4	Lyon Botanic Garden, 000604	KC823011	KC985308	KC985397
<i>Vanda sumatrana</i> Schltr.	Sumatra	1	Gardiner, 126b (K)	KC823012	KC985309	KC985398
<i>Vanda sumatrana</i> Schltr.		2	Gardiner, 106 (K)	KC823013	KC985310	KC985399
<i>Vanda tessellata</i> (Roxb.) W.J.Hook. ex G.Don	China, Assam, Bangladesh, India, Nepal, Sri Lanka, Himalayas, Burma	1	Gardiner, 56 (FLAS) (K)	KC823014	KC985311	KC985400
<i>Vanda tessellata</i> (Roxb.) W.J.Hook. ex G.Don		2	Gardiner, 241 (K)	KC823015	KC985312	KC985401
<i>Vanda tessellata</i> (Roxb.) W.J.Hook. ex G.Don		3	Gardiner, 11 (K)	KC823016	KC985313	KC985402
<i>Vanda tessellata</i> (Roxb.) W.J.Hook. ex G.Don		4	Gardiner, 57 (K)	KC823017	KC985314	KC985403
<i>Vanda tessellata</i> (Roxb.) W.J.Hook. ex G.Don		5	Gardiner, 43 (K)	KC823018	KC985315	KC985404
<i>Vanda tessellata</i> (Roxb.) W.J.Hook. ex G.Don		6	Chase, 17946 (K)	KC823019	KC985316	KC985405
<i>Vanda testacea</i> (Lindl.) Rchb.f.	Assam, Bangladesh, India, Nepal, Sri Lanka, Himalayas, Burma, Thailand		Gardiner, 29 (FLAS) (K)	KC823020	KC985317	KC985406
<i>Vanda tricolor</i> Lindl.	Java, Lesser Sunda Islands	1	913067 (L)	EF655777	KC693487	EF670422
<i>Vanda tricolor</i> Lindl.		2	Chase 17970 (K)	KC823021	KC985318	KC985407

Table 1. Continued

Species	Geographic range	Additional reference number in phylogeny	Voucher/herbarium	<i>matK</i>	<i>psbA</i>	<i>trnL-F</i>
<i>Vanda tricolor</i> var. <i>suavis</i> (Lindl.) Rchb.f. in W.G.Walpers	Java, Lesser Sunda Islands	1	OR-310–1999 (SLZ)	KC823022	KC985319	KC985408
<i>Vanda tricolor</i> var. <i>suavis</i> (Lindl.) Rchb.f. in W.G.Walpers		2	Chase, 19069 (K)	KC823023	KC985320	KC985409
<i>Vanda tricolor</i> var. <i>suavis</i> (Lindl.) Rchb.f. in W.G.Walpers		3	Gardiner, 55 (FLAS) (K)	KC823024	KC985321	KC985410
<i>Vanda tricolor</i> var. <i>suavis</i> (Lindl.) Rchb.f. in W.G.Walpers		4	Chase, 17971 (K)	KC823025	KC985322	KC985411
<i>Vanda ustii</i> Golamco, Claustro & de Mesa	Philippines		Gardiner, 122 (K)	KC823026	KC985323	KC985412
<i>Vanda vietnamica</i> (Haager) L.M.Gardiner, in this study: <i>Christensonia</i> <i>vietnamica</i>	Vietnam	1	200 101 53 (L)	EF655803	KC693489	EF670413
<i>Vanda vietnamica</i> (Haager) L.M.Gardiner, in this study: <i>Christensonia</i> <i>vietnamica</i>		2	Chase, 17943 (K)	KC823027	KC985324	KC985413
<i>Aerides odorata</i> Lour.	China, Assam, Bangladesh, India, Nepal, Himalayas, Andaman Islands, Laos, Burma, Thailand, Vietnam, Borneo, Java, Lesser Sunda Islands, Malaysia, Philippines, Sulawesi, Sumatra		Chase, 15081 (K)	KC823028	KC985325	KC985414

Table 1. *Continued*

Species	Geographic range	Additional reference number in phylogeny	Voucher/herbarium	<i>matK</i>	<i>psbA</i>	<i>trnL-F</i>
<i>Aerides rosea</i> Lodd. ex Lindl. & Paxton	China, Assam, East Himalayas, Laos, Burma, Thailand, Vietnam		Gardiner, 32 (FLAS)	KC823029	KC985326	KC985415
<i>Dimorphorchis graciliscapa</i> (A.L. Lamb & Shim) P.J. Cribb	Borneo		Heidelberg Botanic Garden, 122351	EF655807	KC693493	EF670429
<i>Holcoglossum flavescens</i> (Schltr.) Z.H.Tsi	China		Roberts, s.n. (K)	KC823034	KC985331	KC985420
<i>Holcoglossum kimballianum</i> (Rchb.f.) Garay	Yunnan, Laos, Burma, Thailand		AK010825/1/01 (Z)	EF655783	KC693490	EF670419
<i>Holcoglossum subulifolium</i> (Rchb.f.) Christenson	Hainan, Burma, Thailand, Vietnam		Munich, s.n. (M)	EF670409	KC693491	EF670409
<i>Papilionanthe hookeriana</i> (Rchb.f.) Schltr.	Thailand, Vietnam, Borneo, Malaysia, Sumatra		Gardiner, 54 (K)	KC823035	KC985332	KC985421
<i>Papilionanthe teres</i> (Rchb.f.) Schltr.	China, Assam, Bangladesh, East Himalayas, India, Nepal, Andaman Islands, Laos, Burma, Nicobar Islands, Thailand, Vietnam		Gardiner, 52 (K)	KC823036	KC985333	KC985422
<i>Papilionanthe uniflora</i> (Lindl.) Garay	Assam, East Himalayas, Nepal		Chase, O-1489 (K)	KC823037	KC985334	KC985423
<i>Phalaenopsis cornu-cervi</i> (Breda) Blume & Rchb.f.	Bangladesh, Laos, Burma, Nicobar Islands, Thailand, Vietnam, Borneo, Java, Malaysia, Philippines, Sumatra		Chase, O-1356 (K)	KC823038	KC985335	KC985424
<i>Renanthera isosepala</i> Holttum	Thailand, Borneo		Gardiner, 33 (K)	KC823039	KC985336	KC985425
<i>Renanthera storiei</i> Rchb.f.	Philippines		Gardiner, 37 (K)	KC823040	KC985337	KC985426

Table 1. *Continued*

Species	Geographic range	Additional reference number in phylogeny	Voucher/herbarium	<i>matK</i>	<i>psbA</i>	<i>trnL-F</i>
<i>Rhynchostylis gigantea</i> (Lindl.) Ridl.	China, Cambodia, Laos, Burma, Thailand, Vietnam, Borneo, Malaysia, Philippines		Leiden Botanic Garden, 913013 (L)	AY557202	KC693494	EF670411
<i>Sedirea japonica</i> (Rchb.f.) Garay & H.R.Sweet	Japan, Korea		s.n. (Z)	EF655794	KC693495	EF670433
<i>Seidenfadenia mitrata</i> (Lindl.) Holttum	Burma		AK1035 (Z/ZT)	KC333051	KC333052	KC333053
<i>Taprobanea spathulata</i> (L.) Christenson	India, Sri Lanka		Gardiner, 108 (K)	KC823041	KC985338	KC985427
<i>Vandopsis gigantea</i> (Lindl.) Pfitzer	China, Bangladesh, Laos, Burma, Thailand, Vietnam, Malaysia		GT5512 (C)	KC823042	KC985339	KC985428

Table 2. Primer sequences used to amplify and sequence *matK*, *psbA*, and *trnL-F* regions

Region	Primer	Sequence	Author
<i>matK</i>	19F	CGTTCTGACCATATTGCACTATG	Gravendeel <i>et al.</i> , 2001
	trnK-2R	AACTAGTCGGATGGAGTAG	Johnson & Soltis, 1994
	OMAT396F	CAGAATTTACGATCTATTTCAT	Topik <i>et al.</i> , 2005
<i>psbA-trnH</i>	<i>psbA</i>	GTTATGCATGAACGTAATGCTC	Sang <i>et al.</i> , 1997
	<i>trnH</i>	CGCGCATGGTGGATT CACAATCC	Tate & Simpson, 2003
<i>trn L-F</i>	C	CGAAATCGGTAGACGCTACG	Taberlet <i>et al.</i> , 1991
	F	ATTTGAACTGGTGACACGAG	Taberlet <i>et al.</i> , 1991

including the coding *matK* gene and parts of the neighbouring 5' and 3' *trnK* intron regions. The primers *psbA* (Sang, Crawford & Stuessy, 1997) and *trnH* (Tate & Simpson, 2003) were used to amplify the *psbA-trnH* region (referred to here as '*psbA*'), encompassing the non-coding intergenic spacer region between the two genes coding for the *trnH* tRNA and the gene *psbA*. Other than in eudicots, the *psbA* region also includes the *rps19* gene because of the expansion of the junction between the inverted repeat region 'A' and the large single copy regions (Chang *et al.*, 2006). Primers c and f (Taberlet *et al.*, 1991) were used to amplify the region between the *trnL* (UAA) 5' exon and *trnF* (GAA). The amplicon includes parts of the *trnL* intron, the 3' part of the *trnL* (UAA)

exon and most of the spacer between *trnL* (UAA) 3' and *trnF* (GAA). For the sake of easier readability we refer to this region in the ongoing text as '*trnL-F*'. The polymerase chain reaction (PCR) amplifications were carried out in 50- μ L volumes, using 0.2 μ M of each primer, 5 μ L ammonium (NH₄) buffer (1 \times), magnesium chloride (MgCl₂) (3 mM for *matK*, 2.5 mM for *psbA* and *trnL-F*), 10 mM deoxynucleoside triphosphates (dNTPs), 0.2 μ L *Taq* polymerase (Bioline, 1 unit) and c. 20–70 ng DNA template. PCR amplification was carried out in a DNA Engine Tetrad 2, Peltier Thermal Cycler (MJ Research) using the following reaction profile: 95 °C (2 min for *matK*, 5 min for *psbA*, 1 min for *trnL-F*); 35 cycles of 95 °C for 30 s, 1 min (50 °C for *matK* and *trnL-F*, 48 °C for *psbA*) and

Table 3. Characteristics of *matK*, *psbA* and *trnL-F* DNA sequences, alignments and information content

	Mean length	Shortest length	Longest length	Alignment length	Variable positions	Potentially parsimony-informative characters (PICs)
<i>matK</i>	1714 bp	1554 bp (<i>Rhynchosstylis gigantea</i>)	1753 bp (<i>Papilionanthe uniflora</i>)	1838 bp	192	84
<i>psbA</i>	758 bp	693 bp (<i>Sedirea japonica</i>)	784 bp (<i>Vanda cristata</i>)	819 bp	25	15
<i>trnL-F</i>	1040 bp	859 bp (<i>Aerides rosea</i>)	1295 bp (<i>Vanda ampullacea</i>)	874 bp	58	55
Total alignment	–	–	–	3531 bp	275	154

72 °C for 1 min 40 s; and 72 °C for 5 min. PCR amplified products were purified using QIAquick™ PCR Purification Kit (Qiagen) following the manufacturer's recommended protocol.

Cycle sequencing reactions were performed using Big Dye v.3.1 terminator chemistry (PE Biosystems) for all three regions, with the same primers used for amplification, plus an additional primer to sequence the central region of *matK* (Topik *et al.*, 2005). Sequencing reactions were carried out in 10-µL volumes, with 2 µL Big Dye v.3.1, 1 µL Big Dye buffer (5×), 1 µL primer (3.2 µM for *matK* and *trnL-F*, 10 µM for *psbA*) and 1 µL DNA template (*c.* 20–25 ng). A DNA Engine Tetrad 2, Peltier Thermal Cycler (MJ Research) was used with the following sequencing reaction profile: 96 °C for 1 min; 25 cycles of 96 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min. Sequences were obtained using an automated ABI 3700 capillary sequencer (PE Biosystems). Sequences were assembled and edited using the program Chromas (Technelysium Pty Ltd, 2005).

PHYLOGENETIC ANALYSES

Sequences were aligned using the Clustal X algorithm implemented in the program BioEdit (Hall, 1999), followed by manual optimization with BioEdit or MacClade for OS X (Maddison & Maddison, 2005). Phylogenetic analyses were conducted under maximum parsimony (MP) and maximum likelihood (ML) optimality criteria using PAUP 4.0a123 for Macintosh (Swofford, 2002) for parsimony reconstruction and GARLI v.2.0 (Zwickl, 2006) for ML inference. Settings for the MP heuristic analysis were as follows: tree bisection–reconnection (TBR) branch swapping and MulTrees activated with 100 replicates of random sequence addition. All the analyses interpreted gaps as missing data. Internal support was assessed by non-parametric bootstrapping (Felsenstein, 1985) using the following settings

for PAUP (Müller, 2005: 10 000 bootstrap replicates, a simple-taxon-addition tree as the starting point, TBR swapping and one tree held in memory (MulTrees inactivated). Nodes with bootstraps of 85% are considered strongly supported here, whereas 75–84% is moderately and 0–74% is weakly supported (Chase *et al.*, 2006). The most appropriate model of molecular evolution for each partition was found using the internal model find algorithm of GARLI v2.0, which selected a GTR + I + G model for all partitions. The number of generations for the ML analysis was set at 5 million, with allowance for an automated stop when no new and significantly better topology was detected. A ML heuristic bootstrap analysis (BPML) was performed with 100 replicates to obtain support for nodes.

RESULTS

CHARACTERISTICS OF *matK*, *psbA* AND *trnL-F* DNA SEQUENCES AND INFORMATION CONTENT

Table 3 gives details of the sequence and alignment lengths and information content of the data included in this study. Length variation in the *matK* sequences was mainly caused by the indels found in the *trnK* intron adjacent to the 3' end of *matK*. Edited DNA sequences of *psbA* and *trnL-F* were highly variable in length and many indels were introduced to the aligned sequence matrices in order to accommodate this length variation. In some regions, DNA sequences could not be unambiguously aligned, particularly because of short repeated sequences, and these regions were removed from the analyses of the *matK*, *psbA* and *trnL-F* data sets. In the *matK* and *trnL-F* sequences, short (3 bp) and medium-sized (70 bp; Kocyan *et al.*, 2008) inversions were detected. These were excluded from the analyses or reverse complemented for use in the tree calculations.

PHYLOGENETIC ANALYSES

The phylogenetic trees generated in the combined evidence analyses show a number of well-supported clades, although overall resolution is low in the strict consensus trees produced. We present here only one of the 20 000 most-parsimonious trees, annotated with bootstrap support values > 50% consistent with these topologies (Figs 1, 2), with a total step length of 289, consistency index 0.61, retention index 0.85.

In the analyses there is strong bootstrap support (93% MP, 98% ML) for a *Vanda s.l.* clade, including *Ascocentrum*, *Christensonia* and *Neofinetia*. The *Vanda s.l.* clade plus the three *Holcoglossum* spp. included in the analyses form a clade, with 71% bootstrap support in the MP analysis and 84% support in the ML analysis. The three *Holcoglossum* spp. do not form a monophyletic group, but appear to be paraphyletic to *Vanda s.l.*, the nodes separating them have < 50 BP (bootstrap percentage). In the *Vanda s.l.* clade, resolution of the 'backbone' is extremely poor, with a large polytomy separating lower level clades. Most of these lower level clades receive moderate to high levels of bootstrap support and are considered individually here. The three *Ascocentrum* spp. included do not form a monophyletic group in any of the analyses. *Ascocentrum ampullaceum* (Roxb.) Schltr. and *A. christensonianum* Haager group with high to good support of 80 and 74% in the MP and ML combined analyses, but *A. curvifolium* (Lindl.) Schltr. forms a well-supported clade with the type species for *Vanda*, *V. tessellata*, and *V. cristata* Wall. ex Lindl. and *V. flavobrunnea* Rchb.f. [species formerly placed in their own genus, *Trudelia* (Senghas, 1988b)] with 70 and 75% support. These species (*V. tessellata*, *V. cristata* and *V. flavobrunnea*), form a clade of their own with moderate support, 58 and 63%. A highly supported Philippines clade of *V. lamellata* Lindl., *V. roeblingiana* Rolfe and *V. sanderiana* is resolved with 98 and 97% support. *Christensonia vietnamica*, *V. flabellata* and *V. lilacina* Teijsm. & Binn. form a moderately supported clade, with 71 and 57% support, and *V. brunnea* Rchb.f. and *V. denisoniana* Benson & Rchb.f. form a highly supported group with 96% support in both the MP and ML combined analyses. *Vanda coerulea* and *V. coerulescens* Griff. form a moderately well-supported group with 80 and 70% support in the combined analyses and *V. bensonii* Bateman, *V. liouvillei* Finet and *V. testacea* (Lindl.) Rchb.f. form a moderately to highly supported clade with 82 and 89% support.

The combined analyses resolve a large, moderately to highly supported, 'South-East Asian archipelago' clade with 82 and 89% support, comprising *V. arcuata* J.J.Sm., *V. celebica* Rolfe, *V. dearei* Rchb.f., *V. devoogtii* J.J.Sm., *V. furva* (L.) Lindl., *V. hastifera*

Rchb.f., *V. helvola* Blume, *V. hindsii* Lindl., *V. insignis* Blume, *V. jennae* P.O'Byrne & J.J.Verm., *V. limbata* Blume, *V. lindenii* Rchb.f., *V. luzonica* Loher ex Rolfe, *V. merrillii* Ames & Quisumb., *V. metusalae* P.O'Byrne & J.J.Verm., *V. perplexa* Motes & D.L.Roberts, *V. sumatrana* Schltr., *V. tricolor* Lindl. and *V. ustii* Golamco, Claustro & de Mesa. In this clade, most relationships between taxa are unresolved, but several clades receive moderate to high support. *Vanda limbata* and *V. perplexa* form a highly supported clade, with 95 and 98% support in the combined analyses. *Vanda celebica*, *V. furva* and *V. lindenii* form a moderately supported clade, with 63 and 68% support. A moderately well-supported smaller clade, comprising *V. arcuata*, *V. dearei*, *V. devoogtii*, *V. helvola*, *V. hindsii*, *V. insignis*, *V. jennae*, *V. luzonica*, *V. merrillii*, *V. metusalae*, *V. sumatrana*, *V. tricolor* and *V. ustii*, is resolved in the combined analyses, with 63 and 59% support. Outside the *Vanda s.l.* and *Holcoglossum* clade, the three *Papilionanthe* spp. form a clade with 100% support, the two *Aerides* spp. form a clade with 88%, the two *Renanthera* spp. form a clade with 93% and *Aerides*, *Renanthera* and *Taprobanea* form a clade with 89%.

DISCUSSION

MOLECULAR CHARACTERISTICS AND INFORMATION
CONTENT OF THE VANDA DATA

The molecular data obtained here contain a relatively low level of phylogenetic information. This is congruent with the findings of Kocyan *et al.* (2008), who found that the *matK* sequences that they obtained from *Aerides* contained 94 potentially parsimony informative characters, but the *Vanda* sequences only contained 80. In the overall Aeridinae study of Topik *et al.* (2005), > 400 parsimony informative characters were identified, with almost twice as many in the *Aerides trnL-F* sequences than in the *Vanda trnL-F* sequences (98 vs. 54 parsimony informative characters). The often long and hypervariable P8 region in the *trnL* intron appears to be insignificant in *Vanda*, contrasting with the region in *Aerides* and other angiosperms, which often exhibits extreme length variability (Borsch *et al.*, 2003; Kocyan *et al.*, 2008). Another feature of the *trnL* intron in vandaceous taxa is the relatively frequent occurrence of a 70-bp inversion in the P8 region. In Aeridinae, this inversion appears in several unrelated genera and this region must either be reverse complemented or excluded from analysis or tree search algorithms result in incorrect phylogenetic reconstructions (Kocyan *et al.*, 2008; A. Kocyan, unpubl. data). However, in the data set presented here, the inversion was only detected in

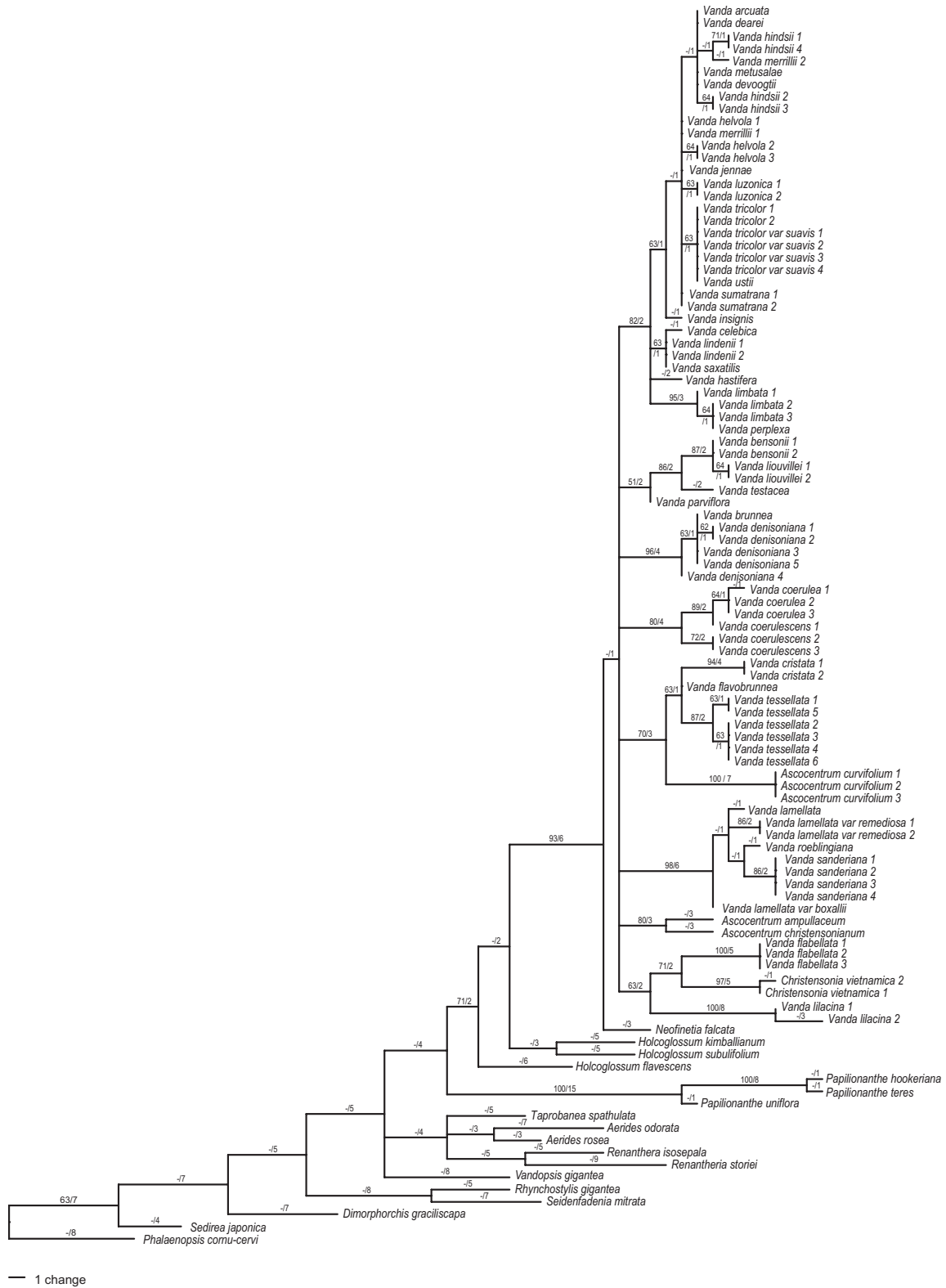


Figure 1. One of the 20 000 most-parsimonious trees obtained using the combined plastid DNA data set and maximum parsimony, showing bootstrap values over 50% (first number above branches) and branch lengths (second number).

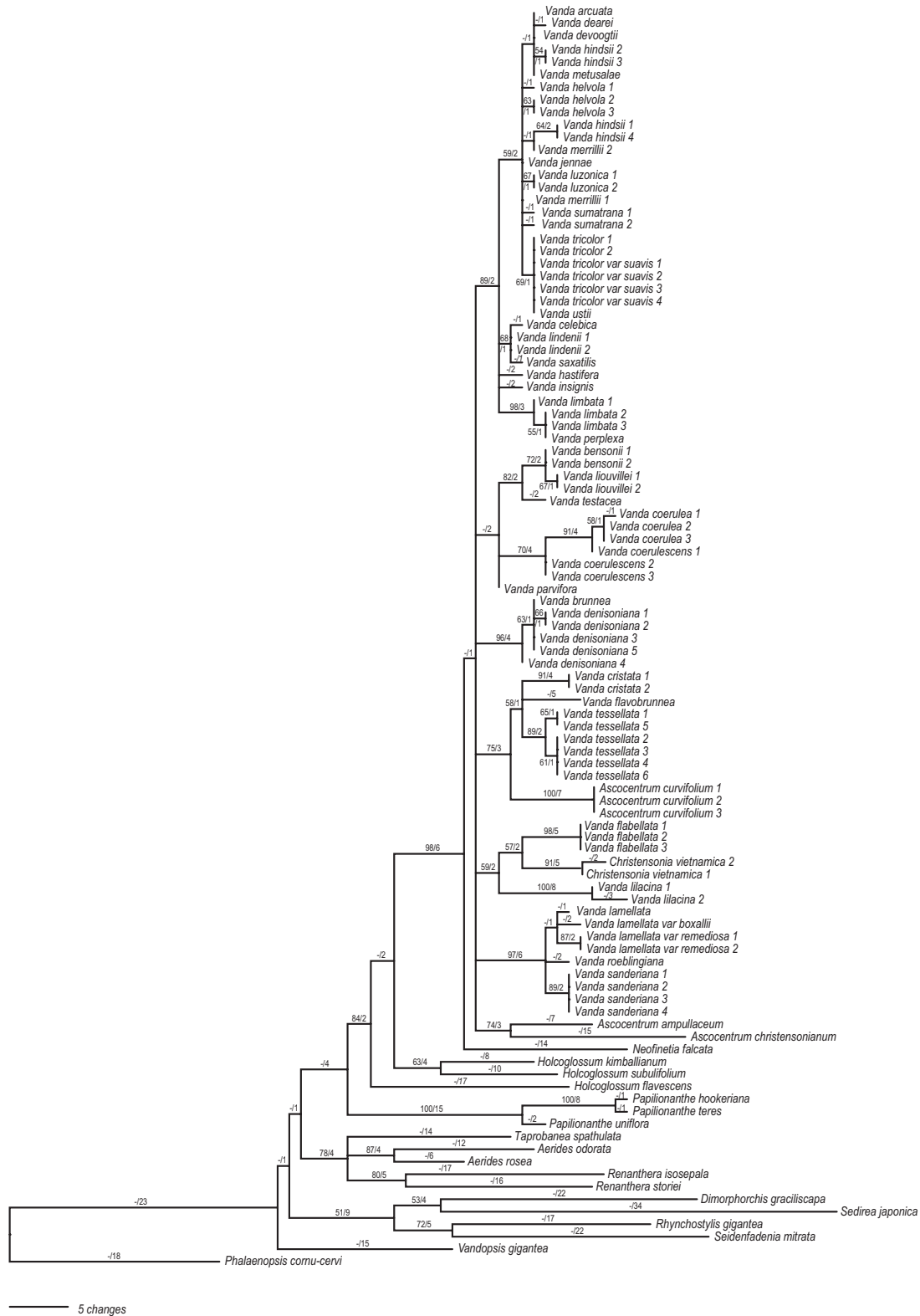


Figure 2. One of the most likely trees obtained using the combined plastid DNA data set and maximum likelihood, showing bootstrap values over 50% (first number above branches) and branch lengths (second number).

the *Seidenfadenia mitrata* (Rech.f.) Garay sequence, not in any of the taxa of *Vanda s.l.*

RELATIONSHIPS OF *VANDA* TO GENERA OF OTHER AERIDINAE

Our analyses consistently resolve a *Vanda s.l.* clade with high levels of support, including *Ascocentrum*, *Neofinetia* and the monotypic *Christensonia*. Recently, in as yet unpublished analyses to be incorporated into volume 6 of the *Genera Orchidacearum* series (Pridgeon *et al.*, 2014), Kocyan produced the most complete phylogeny of Aeridinae to date, comprising 199 species in 98 genera, and using three plastid DNA regions and one nuclear region (A. Kocyan, unpubl. data). In that analysis, Kocyan showed that *Vanda*, *Ascocentrum* and *Christensonia* form a highly supported clade (99% bootstrap support), and the larger grouping of this clade plus *Ascocentropsis* Senghas & Schildh., *Eparmatostigma* Garay and *Neofinetia* similarly receives high support (99% bootstrap support). In neither piece of research does *Vanda s.l.* clearly fall into well-supported clades corresponding with any of the existing genera with more than one species. As a result, we support the inclusion of the genera *Ascocentrum* and *Neofinetia* in *Vanda*. The retention of *Ascocentropsis*, *Christensonia* and/or *Eparmatostigma* would require the splitting of *Vanda*. This cannot be carried out with any logic or consistency on the basis of these results. Instead, the most practical solution is to include these small genera in *Vanda* and publish the appropriate new combinations of names in *Vanda* (those which have not already been made by previous authors) (Gardiner, 2012; 2014; Gardiner & Cribb, 2013). Figure 3 shows a range of the floral diversity in the newly expanded concept of the genus *Vanda*.

Although only three of nine species of *Ascocentrum* were included in these analyses, the genus was not resolved as monophyletic in any of the analyses performed. The results of Xiang *et al.* (2012), which included five *Ascocentrum* spp., otherwise focusing on *Holcoglossum*, similarly did not find *Ascocentrum* to be monophyletic in any of their analyses. Four of the included species grouped with *Vanda*, *Neofinetia* and *Aerides*, and the fifth species, *Ascocentrum himalaicum* (Deb, Sengupta & Malick) Christenson, nested with their main data set of 13 *Holcoglossum* spp. Non-parametric bootstrapping of a smaller earlier *Vanda s.l.* data set (Gardiner, 2008) could not provide support for the hypothesis of the monophyly of *Ascocentrum* and, in spite of distinct morphological characters for these species (supporting their maintenance as a separate genus), it appears that the genus as it stands is polyphyletic and the species should be incorporated into the wider concept of *Vanda*.

Congruent with the results of Xiang *et al.* (2012), *Holcoglossum* is shown here to be the most likely sister genus to *Vanda s.l.*, although, with limited sampling of the genus, the three species included appear to be paraphyletic to the *Vanda s.l.* clade. Potentially *Holcoglossum* should also be incorporated into *Vanda s.l.* In the absence of additional taxa, we prefer to leave this morphologically distinctive genus separate for the time being, with its semi-terete leaves and floral column foot, especially as it does not appear to nest in *Vanda s.l.* The unpublished results of Kocyan also reveal *Holcoglossum* to be the sister genus to *Vanda s.l.*, although in these analyses the genus is resolved as a monophyletic sister group, rather than being paraphyletic to *Vanda s.l.* Similarly, Fan *et al.* (2009) found a sister relationship between *Holcoglossum* and a clade comprising four species of *Vanda*, *Aerides flabellata*, *Ascocentrum ampullaceum* and *Neofinetia falcata*. The monotypic *Taprobanea spathulata* (L.) Christenson, previously placed in *Vanda* by Sprengel (1826), is shown in all analyses to fall well outside of the *Vanda s.l.* clade and is not considered to be a member of, or closely related to, the genus.

RELATIONSHIPS WITHIN *VANDA* AND BETWEEN SECTIONS

Within the genus *Vanda s.l.*, although the main 'backbone' of the genus is unresolved, there are a number of lower level clades that receive moderate to high levels of bootstrap support. These clades are not congruent with existing morphological classifications of *Vanda*, but morphological characters can be attributed to most, in line with a proposed new classification of *Vanda* (Gardiner & Cribb, 2013; M. Motes and L. M. Gardiner, unpubl. data). The morphological sections proposed are shown in Table 4 and are represented on the ML tree shown in Figure 4. Furthermore, there appears to be evidence of some degree of biogeographic signal in our data. Section *Roeblingiana* Motes & W.E.Higgins is a well-supported group of species centred geographically in the Philippines (*V. barnesii* W.E.Higgins & Motes, *V. javierae* D.Tiu ex Fessel & Lückel, *V. lamellata*, *V. roeblingiana* and *V. sanderiana*), and the large, well-supported but unresolved group of species representing sections *Deltaglossa* Christenson and *Dactylobata* W.Suarez & J.Cootes are all from the South-East Asian archipelago, with only short branch lengths separating the species. The remaining groups of species seen in the phylogenetic trees produced are distributed in mainland Asia and Indochina.

POLLINATION AND SPECIATION AMONG *VANDA S.L.*

As in many epiphytic orchids (but see recent reports, e.g. Arditti *et al.*, 2012; Neubig *et al.*, 2012, and ref-

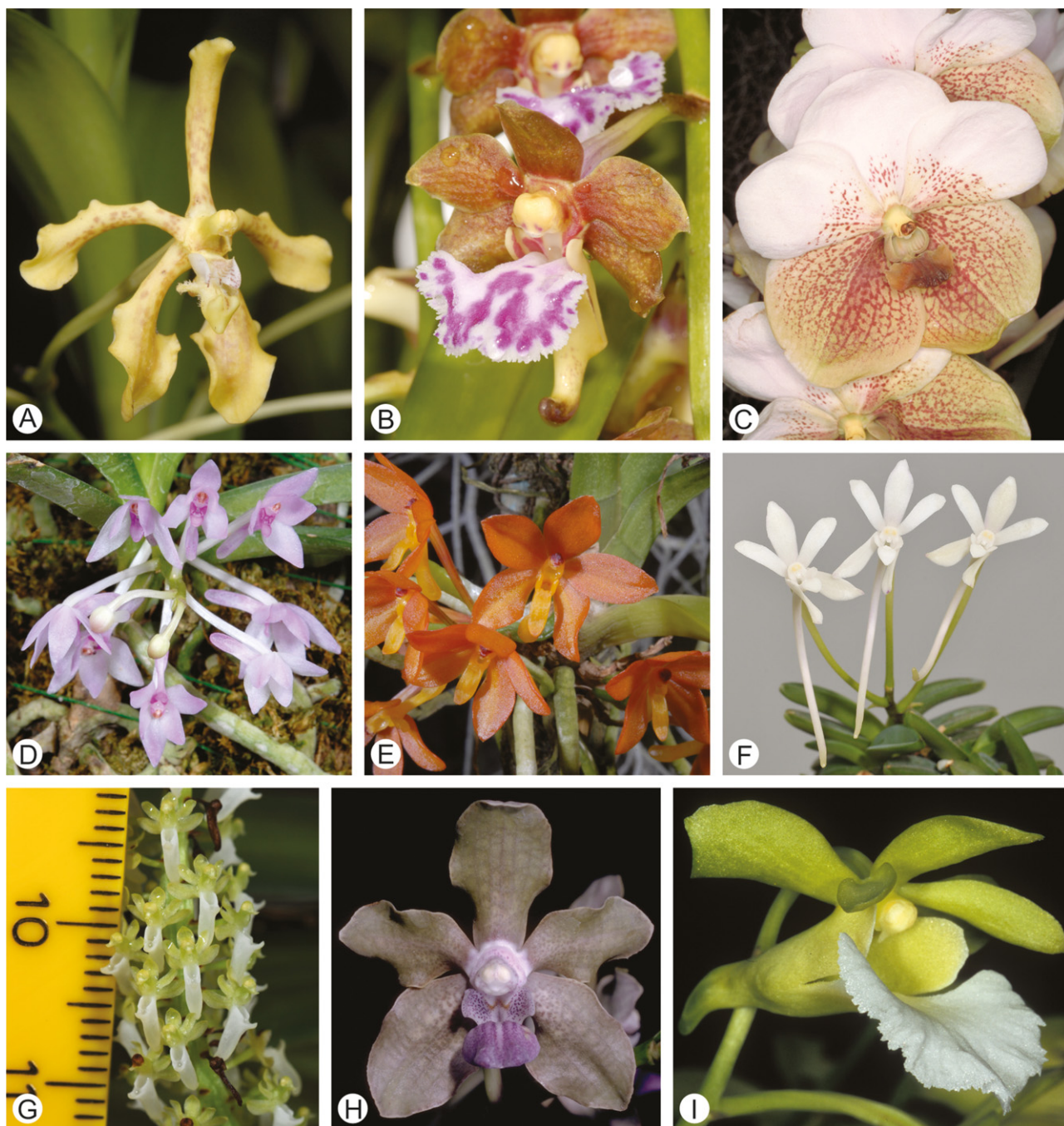


Figure 3. Selected taxa of *Vanda* representing the floral diversity of the genus. A, *Vanda scandens* Holttum. B, *Vanda flabellata*. C, *Vanda sanderiana*. D, *Vanda nana* L.M.Gardiner. E, *Vanda ampullacea* var. *aurantiaca*. F, *Vanda falcata*. G, *Vanda dives* (Rchb.f.) L.M.Gardiner. H, *Vanda tessellata*. I, *Vanda vietnamica*. Scale bar, minor divisions are equal to 1 mm (G). Photograph credits: A, B, D, E, G, I. Alexander Kocyan; C. Peter O'Byrne; F. Rogier van Vugt; H. Alan Hoffman.

erences therein), there is an almost complete lack of pollinator information for the now extended concept of *Vanda*. Many *Vanda* spp. with large, showy, colourful flowers, often with open, spreading tepals, are visited by bees and butterflies in cultivation, and

Holttum (1953) concluded that 'typical' *Vanda* spp. are pollinated by bees. Melitophily (bee pollination) and cantharophily (beetle pollination) seem to be the only pollination systems for which we have a few reports. Pradhan (1983) observed beetles as efficient

Table 4. Overview of the morphological sections in *Vanda* proposed by Gardiner & Cribb (2013) and M. Motes and L. M. Gardiner, unpubl. data

Proposed section/ group	Species	Key morphological characters	Geographical distribution
<i>Vanda</i> (eight species)	<i>V. bidupensis</i> Aver. & Christenson <i>V. concolor</i> Blume <i>V. fuscoviridis</i> Lindl. <i>V. motesiana</i> Choltco <i>V. stangeana</i> Rchb.f. <i>V. subconcolor</i> Tang & F.T.Wang <i>V. tessellata</i> (Roxb.) W.J.Hook. ex G.Don <i>V. wightii</i> Rchb.f. in W.G.Walpers	<ul style="list-style-type: none"> • Small side lobes on labellum, sometimes pointed in shape • Fleshy labellum • Rounded column 	Mainland Asia and adjacent islands, Sri Lanka, Indian subcontinent, Himalayas, southern China, Indochina
<i>Cristatae</i> (seven species)	<i>V. alpina</i> (Lindl.) Lindl. <i>V. chlorosantha</i> (Garay) Christenson <i>V. cristata</i> Wall. ex. Lindl. <i>V. flavobrunnea</i> Rchb.f. <i>V. griffithii</i> Lindl. <i>V. longitepala</i> D.L.Roberts, L.M.Gardiner & Motes <i>V. thwaitesii</i> Hook.f. in H.Trimen	<ul style="list-style-type: none"> • Fleshy labellum • Labellum with minimal spur • Tepals yellow–green in colour • Often with deep red/purple markings on labellum 	Himalayas, one species in Indochina
<i>Longicalcarata</i> (two species)	<i>V. coerulea</i> Griff. ex. Lindl. <i>V. coerulescens</i> Griff.	<ul style="list-style-type: none"> • Blue coloration to tepals and labellum • Long flower spikes, sometimes branched • Compact plants • Distinctly spurred labellum 	Himalayas, India, Nepal, Bhutan, China, Indochina
<i>Testacea</i> (four species)	<i>V. bensonii</i> Batem. <i>V. liouvillei</i> Finet. <i>V. parviflora</i> Lindl. <i>V. testacea</i> (Lindl.) Rchb.f.	<ul style="list-style-type: none"> • Blue coloration to tepals and labellum • Long flower spikes, sometimes branched • Compact plants • Distinctly spurred labellum 	Indian subcontinent, Indochina
<i>Obtusiloba</i> (four species)	<i>V. bicolor</i> Griff. <i>V. brunnea</i> Rchb.f. <i>V. denisoniana</i> Benson & Rchb.f. <i>V. vipanii</i> Rchb.f.	<ul style="list-style-type: none"> • Orbicular side lobes on labellum • Large, fleshy labellum • Labellum often pandurate • Minimal spur on labellum • Labellum attached to column foot 	Indochina and adjacent Himalayas
<i>Flabellata</i> (three species)	<i>V. flabellata</i> (Rolfe ex Downie) Christenson <i>V. lilacina</i> Teijsm. & Binn. <i>V. vietnamica</i> (Haager) L.M.Gardiner	<ul style="list-style-type: none"> • Orbicular side lobes on labellum • Large, fleshy labellum • Labellum often pandurate • Minimal spur on labellum • Labellum attached to column foot 	Indochina
<i>Deltoglossa</i> (17 species)	<i>V. arcuata</i> J.J.Sm. <i>V. deareii</i> Rchb.f. <i>V. devoogtii</i> J.J.Sm. <i>V. foetida</i> J.J.Sm. <i>V. helvola</i> Blume <i>V. hindsii</i> Lindl. <i>V. insignis</i> Blume <i>V. jennae</i> O'Bryne & Verm. <i>V. limbata</i> Blume <i>V. lumbokensis</i> J.J.Sm. <i>V. luzonica</i> Loher ex Rolfe <i>V. merrillii</i> Ames & Quisumb. <i>V. metusalae</i> O'Byrne <i>V. perplexa</i> Motes & D.L.Roberts <i>V. sumatrana</i> Schltr. <i>V. tricolor</i> Lindl. <i>V. ustii</i> Golanco, Claustro & de Mesa	<ul style="list-style-type: none"> • Cylindrical column with thickened base 	Indonesian and Philippine archipelagos

Table 4. Continued

Proposed section/ group	Species	Key morphological characters	Geographical distribution
<i>Dactylobata</i> (seven species)	<i>V. celebica</i> Rolfe <i>V. furva</i> (L.) Lindl. <i>V. gibbsiae</i> Rolfe <i>V. hastifera</i> Rchb.f. <i>V. lindenii</i> Blume <i>V. frankieana</i> Metusala and O'Byrne <i>V. scandens</i> Holttum	• Distinct appendages (lobules) on labellum mid lobe	Island endemics in Indonesian archipelago
<i>Roeblingiana</i> (five species)	<i>V. barnesii</i> W.E.Higgins & Motes <i>V. javierae</i> D.Tiu ex Fessel & Lückel <i>V. lamellata</i> Lindl. <i>V. roeblingiana</i> Rolfe <i>V. sandieriana</i> (Rchb.f.) Rchb.f.	• Cylindrical column without thickened base • Usually modified/embellished labellum mid lobe	Philippines and adjacent islands
<i>Ascocentrum</i> (nine species)	<i>V. ampullacea</i> (Roxb.) L.M.Gardiner <i>V. aurantiaca</i> (Schltr.) L.M.Gardiner <i>V. aurea</i> (J.J.Sm.) L.M.Gardiner <i>V. christensoniana</i> (Haager) L.M.Gardiner <i>V. curvifolia</i> (Lindl.) L.M.Gardiner <i>V. garayi</i> (Christenson) L.M.Gardiner <i>V. insularum</i> (Christenson) L.M.Gardiner <i>V. miniata</i> (Lindl.) L.M.Gardiner <i>V. rubra</i> (Lindl.) L.M.Gardiner	• Diminutive size and compact growth habit • Small brightly coloured flowers • Nectar-filled spur • Putatively bird pollinated	Himalayas, southern China, Indochina, through to Philippine and Indonesian archipelagos
<i>Ascocentropsis</i> (three species)	<i>V. himalaica</i> (Deb, Sengupta & Malick) L.M.Gardiner <i>V. nana</i> L.M.Gardiner <i>V. semiteretifolia</i> (Seidenf.) L.M.Gardiner	• Purple flowers • Small brightly coloured flowers – thought to be bird pollinated	Vietnam
<i>Neofinetia</i> (three species)	<i>V. falcata</i> (Thunb.) Beer <i>V. richardsiana</i> (Christenson) L.M.Gardiner <i>V. xichangensis</i> (Z.J.Liu & S.C.Chen) L.M.Gardiner	• White flowers • Long nectar-filled spur • Moth pollinated	China, Japan, eastern Asia
<i>Eparmatostigma</i> (one species)	<i>Vanda dives</i> (Rchb.f.) L.M.Gardiner	• Compact plants • Very numerous minute white flowers on arching raceme	Laos, Vietnam

pollinators on *V. cristata*, and beetles may also pollinate *V. denisoniana* (Christenson, 1992). However, using the concept of floral syndromes on the now extended concept of *Vanda*, we may be able to distinguish four groups of animals as putative pollinators. Bees, in particular *Xylocopa* bees, may play an important role in pollination for most of what was previously considered to be *Vanda s.s.* and *Christensonia*. Beetles may be the main pollinators for the species formerly separated as *Trudelia* (*Vanda* section *Cristatae*). Bird pollination may be likely in the species in section *Ascocentrum* because of the red–orange colouring of the flowers and short, nectariferous spurs [one report exists of a hybrid of section *Ascocentrum* being pollinated by a honey-eater bird in cultivation

(van der Cingel, 2001)], and *V. ampullacea* has violet-coloured pollinia, which are thought to facilitate pollination by birds (which are thought to be less likely to see and therefore remove violet pollinia when self-grooming) (M. Motes, pers. observ.), although butterfly pollination is also possible. Section *Ascocentropsis* appears to exhibit the same pollination syndromes as section *Ascocentrum*. The night-scented white flowers of species of section *Neofinetia* are probably visited by night-active long-tongued moths searching for nectar in the long, narrow spurs of the flowers. Section *Eparmatostigma* also has small white flowers with distinct spurs that contain nectar (A. Kocyan, pers. observ.) and pollination may be by small bees or possibly butterflies. The inclusion of the florally

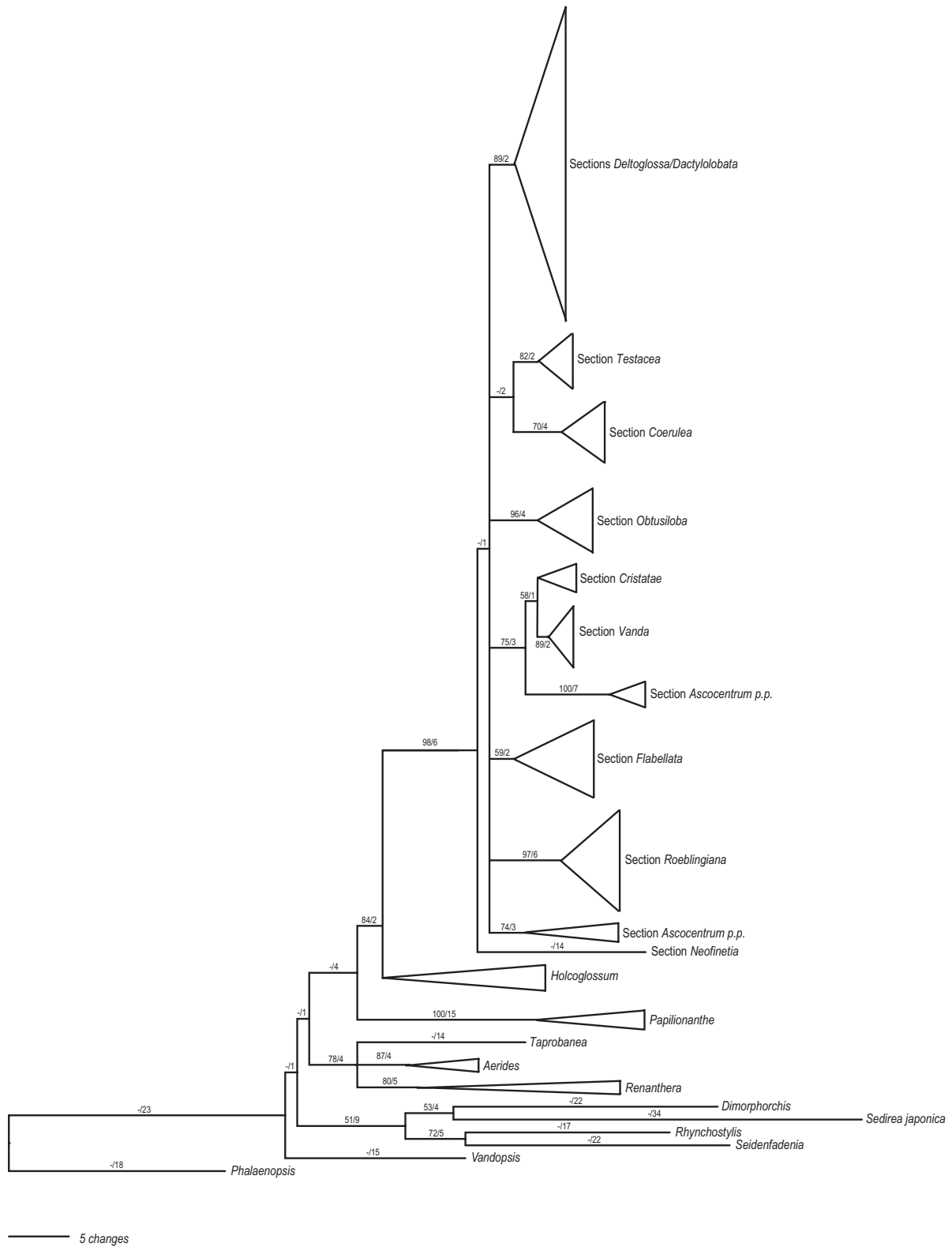


Figure 4. Simplified maximum likelihood tree of the combined plastid analysis, showing the proposed morphological sections in *Vanda*.

distinct genera *Eparmatostigma* and *Neofinetia* into *Vanda s.l.* (as sections *Eparmatostigma* and *Neofinetia*) supports the theory of pollinator-driven selection of floral forms allowing a relatively easy switch between pollination strategies (Kocyan *et al.*, 2008; Sapir & Armbruster, 2010). Moreover, it is unknown whether or not most taxa are nectariferous, and it is possible that a substantial proportion of the species in *Vanda s.l.* are nectarless, as pollinator deceit seems to be one of the key factors in orchid speciation (Cozzolino & Widmer, 2005).

Molecular data suggest that the taxa in *Vanda s.l.* exhibit remarkably low genetic divergence from each other, in spite of the dramatic morphological variation seen across the genus and the broad geographical range of *Vanda s.l.* Low genetic diversity may suggest a relative recent origin of the group under investigation (Bateman, 1999) and, as such, *Vanda* may offer an interesting example of relatively rapid speciation and morphological diversification in response to pollination syndromes and allopatry in insular environments. This may be particularly strongly seen in the South-East Asian archipelago, where morphological diversity appears to be at its highest, and yet genetic divergence is at its lowest. The history of sea levels during the Pleistocene is well documented (Voris, 2000) and it is possible that during these relatively recent events some species became locally extinct, with others produced through speciation processes. A series of periods of low sea levels caused by glaciations during the Pleistocene resulted in a continuous landmass from Borneo to Malaysia–Thailand (Sundaland), and may help to explain the distributions patterns of certain taxa. For example, *V. helvola* occurs in Borneo and Peninsular Malaysia, whereas other species from the same section (section *Deltaglossa*, e.g. *V. dearei*) occur exclusively on Borneo. The first species may have its origin while the land masses were connected, whereas the latter could have originated in Borneo after the isolation of the island.

CONCLUSIONS AND FUTURE DIRECTIONS

It is clear from this study that *Vanda s.l.* remains an enigmatic genus, with unresolved relationships between its species, and a number of small genera having been incorporated from around its periphery. Our plastid-only data set results in relatively poor resolution in the genus and is insufficiently informative to resolve the backbone of the genus clearly and to be able to interpret the phylogeny along biogeographic lines. Nonetheless, the plastid data are sufficiently variable to show the monophyly of this expanded concept of *Vanda*, to support the inclusion of *Ascocentrum*, *Christensonia*, *Euanthe*, *Neofinetia* and *Trudelia* and to support *Holcoglossum* as the

sister genus to *Vanda*. The addition of further plastid regions, or a reliably amplified, low-copy number, phylogenetically informative nuclear marker would be likely to add clarity to these results. Concerted efforts to amplify ITS sequences (the nuclear marker most commonly used in orchid phylogenetics) from *Vanda* spp. have been hampered in the past by the presence of multiple copies of the region, necessitating cloning of samples and complicating the interpretation of results obtained. The problem of multiple copies of ITS may be attributable to the well-reported and discussed incomplete-concerted evolution tendency of the region (e.g. Xiao, Möller & Zhu, 2010), but it also could be influenced by the exceptional variation of chromosome numbers among *Vanda s.s.* and therefore different ploidies; the basic chromosome number of Aeridinae is $x = 19$, which is relatively stable for the majority of the subtribe, but in *Vanda s.s.* published reports are $N = 19, 38$ and 57 (Felix & Guerra, 2010). Recent innovations in using nuclear regions such as the low-copy nuclear *Xdh* gene in orchid phylogenetics (Górniak, Paun & Chase, 2010; A. Kocyan, unpubl. data) and adding more plastid regions (e.g. Ebert & Peakall, 2009) offer promising prospects of furthering this research. Comparing the tree structure of plastid and nuclear data would also allow the search for topological conflicts between data sets and possibly answer questions on the reported hybrid origin of certain taxa (e.g. *Vanda* × *amoena* O'Brien, *V.* × *boumaniae* J.J.Sm., *V.* × *charlesworthii* Rolfe and *V.* × *confusa* Rolfe) and, with that, raising questions about plastid capture and introgression in *Vanda* as recently applied in the genus *Alocasia* (Schott) G.Don in R.Sweet in Araceae (Nauheimer, Boyce & Renner, 2012). In addition, with a more resolved phylogenetic tree for this group, and with the recent re-evaluation of the age of Orchidaceae as a family and increasingly reliable geological reconstructions of the main diversity centres in South-East Asia, an estimation of the age of the genus should be possible and assessed in relation to the biogeographic distributions of the species and land changes at the time of their evolution (Hall, 2001; Gustafsson, Verola & Antonelli, 2010).

Traditional sectional delimitation has influenced the sampling scheme of the research presented here; however, our results do not support these sections, which has unfortunately had an effect on a rather uneven sampling strategy. More effort should be expended on ensuring that all of the newly proposed morphological sections are well sampled in any future molecular work on this group of taxa, as well as including as many of the taxa formerly classified in separate genera as possible (sections *Ascocentropsis*, *Ascocentrum*, *Eparmatostigma* and *Neofinetia*). It is hoped that future sampling will concentrate on

incorporating more taxa from the under-represented groups in order to better elucidate their true relationships to each other.

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