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Molecular Phylogeny and Deep Origins of the Hybrid *Mokara* Dear Heart (Orchidaceae)

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Abstract

Hybridization has played a significant influence in the evolution of plants. The hybrids orchid genus *Mokara* Dear Heart is a cut orchid with beautiful flowers, and it is economically important. Both maximum likelihood (ML) and Bayesian inference (BI) methods were used to construct the phylogeny of *Mokara* Dear Heart to explore the phylogenetic relationships of *Mokara*Dear Heart and its parents based on the molecular data from ITS and *matK* sequences. Our molecular results supported that *Mokara* Dear Heart is closely related to the genus *Vanda*, but far from *Arachnis*. The genetics of chloroplast a type of the plastid and nucleus of *Moraka* Dear Heart could be derived from the *Vanda* lineage. The hybrid orchid *Mokara* Dear Heart is a bigeneric hybridization and originated from *Arachnis* and *Vanda*. In the hybridization scenario of *Mokara* Dear Heart, the *Vanda* Yip Sum Wah acted as the father's parent and crossed with *Arachnis* Maggie Oei's maternal lineage, resulting in paternal transmission of the chloroplast to the *Mokara* Dear Heart. The commercial name *Mokara* Dear Heart should be corrected to *Aranda* Dear Heart.

Keywords: molecular phylogeny, hybrid, chloroplast, nucleus, *Vanda*, *Mokara* Dear Heart, *Aranda* Dear Heart

1. Introduction

Exploring the molecular phylogenetic relationship between taxa can help scientists discover novel findings. Plant evolution has been aided by hybridization, and imperfect lineage sorting is believed to have occurred during several rapid radiations. As a result, there are several examples of accordance and discordance between phylogenetic trees from chloroplast and nuclear genes in plants. Much of the backbone phylogeny of angiosperm has been resolved using a molecular phylogenetic approach (see Soltis *et al*., 2009, 2011; Ruhfel *et al*., 2014) as well as clarified longstanding questions in relationships of major clades of the plant.

Orchidaceae is the largest angiosperm family, with almost 25000 species and 800 genera (Chen *et al*., 2009). The family members were distributed across the whole world except Antarctica (Chen *et al*., 2009; Zhang *et al*., 2013). The species of Orchidaceae play a significant role in economy, ornamental, and medicine. In several tribes of Orchidaceae, Vandeae is a large horticulturally important group with almost 2000 species. *Mokara* the "Smile Orchid", is popularin Asia, where it has been first hybridized and cultivated (Soon, 1989; Dalayap *et al*., 2011). *Mokara* is Vandaceous orchid, which is the result of the trigeneric hybridization of *Ascocentrum*, *Arachnis*, and *Vanda* genera (Dalayap *et al*., 2011; Peyachoknagul *et al*., 2014). Currently, several varieties of *Mokara* were produced with unique and highly variable star-shaped flowers, and flowers with a large number of colors compared to other orchids such as pink, purple, blue, yellow, orange, and red (Dalayap *et al*., 2011). The *Mokara* species are cut orchids with beautiful flowers, and they are economically important. The economic *Mokara* Dear Heart is an orchid hybrid that was registered by Mizuta in 1972 (Woo and Nakamoto, 1990). However, the *Mokara* Dear Heart lineage was hybridized from *Arachnis* Maggie Oei and *Vanda* Yip Sum Wah, in which *Arachnis* Maggie Oei is the seed parent and *Vanda* Yip Sum Wah pollen parent (https://orchidroots.com). Moreover, the accepted name of this lineage was corrected to *Aranda* Dear Heart. Therefore, there are disagreements over the commercial and scientific names for this lineage, which can be resolved by molecular phylogeny.

Several molecular phylogenetic studies for Vandeae members were performed (Carlsward *et al*., 2006; Gardiner *et al*., 2013; Zhang *et al*., 2013; Szlachetko *et al*., 2014; Zou *et al*., 2015). In these studies, the three genera *Ascocentrum*, *Vanda*, and *Arachnis* were included to investigate the phylogenetic relationship and monophyly of the three genera. However, the phylogenetic position and molecular relationship of the hybrids orchid *Mokara* are absent.

Lee (1994) conducted a study on the genetics of *Mokara*, and the results of the study indicated that the lineages of *Mokara* have genetic diversity. The triploid *Mokara* cultivars generally outperform their diploid counterparts in various horticultural characteristics and are more desirable for commercial cut-flower production.

The monophyly of Vandeae was investigated by Carlsward *et al*. (2006) using molecular data from ITS,

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*trn*L-F, and *mat*K; however, the samples of *Mokara*'s three parents are limited. Gardiner *et al*. (2013) presented a molecular study using a sequence of three DNA regions. Results of the study showed that the genus *Vanda* s.l. forms a clade including approximately 73 species, containing some members of *Euanthe*, *Christensonia*, *Ascocentrum*, *Neofinetia*, and *Trudelia*, and the species *Aerides flabellata*. However, the genetic relationships within *Vanda* s.l. remain a mystery, and morphological classifications for the species are inconsistent with the findings.

Zhang *et al*. (2013) elucidated the molecular relationship between 14 genera of the *Aerides*–*Vanda*clade. The *Aerides*–*Vanda* clade was supported as a monophyletic group. The molecular data from five plastid DNA and ITS regions proved that *Ascocentrum* is non-monophyletic. *Pendulorchis* was treated as a new genus; some other treatments were provided for the *Aerides*–*Vanda* clade.

Recently, the close relationship between *Vanda* and *Ascocentrum* was reconstructed by Zou *et al*. (2015) based on five DNA regions (*atp*I-H, *mat*K, *psb*A-*trn*H, *trn*L-F, and ITS) of 211 individuals from 74 genera. In this study, the subtribe Aeridinae was supported as monophyletic, and 10 major clades were recognized in the Aeridinae. Additionally, within the Aeridinae, most genera were strongly supported as monophyletic, and only several genera were found to be polyphyletic.

Thus, in order to investigate the phylogenetic position and molecular relationship between *Aranda*, *Mokara*, and its parents, we conducted the phylogenetic analyses using molecular data from chloroplast and nuclear DNA regions. Our major aims are to (1) construct the phylogenetic placement of *Mokara* Dear Heart and (2) investigate the patterns of phylogenetic relationship consistent with the hybridization of *Mokara* Dear Heart.

2. Materials and Methods

2.1. Taxon sampling

To determine the phylogenetic position of *Mokara* Dear Heart, we assembled sequences of all the three parents genera *Ascocentrum*, *Vanda*, and *Arachnis* that are available in GenBank (NCBI). The duplication and uncertain sequences were excluded. For the *Vanda* genus, all phylogenetic clades of the genus were included following Gardiner *et al*. (2013). In total, 113 sequences that represented 84 species for both ingroups and outgroups were included in the molecular analyses. Two DNA regions including ITS, and *mat*K were used for molecular analyses.

We sampled three individuals of *Mokara* Dear Heart that were collected from Vietnam. The *Mokara* Dear Heart samples were obtained from a nursery of Hanoi Pedagogical University 2. Our *Mokara* Dear Heart samples belong to the *Aranda* variety of *Mokara*. *Mokara* Dear Heart samples were planted in pots of sand in growth chambers. (http://www.orchidsasia.com/mhyblist.htm).

2.2. DNA extraction, polymerase chain reactions (PCR), and sequencing

For the three individuals of *Mokara*Dear Heart collected from Vietnam, we extracted genomic DNA from silica gel dried leaves using the CTAB procedure as previously described (Raskoti and Ale, 2021), and the i-genomic Plant DNA extraction mini kit (iNtRON), following the manufacturer's recommended protocol. The PCR and sequencing were conducted using the primers presented by Gardiner *et al*. (2013). PCR products were separated and visualized using an ABI3730 automated sequencer (Applied Biosystems, USA). All sequences were aligned in Geneious v.8.0.5 (Kearse *et al*., 2012). Voucher information and GenBank accession numbers are listed in AppendixS1. To test the topological incongruence between the nuclear and plastid DNA regions, the incongruence length difference (ILD) test was conducted (Farris *et al*., 1995). The results of the ILD test showed no significant incongruence between the nuclear and the plastid datasets in this study. Consequently, we concatenated them into one dataset.

2.3. Phylogeneticanalyses

The molecular phylogenetic analyses of *Mokara* Dear Heart were performed using both the Bayesian inference (BI) and Maximum likelihood (ML) methods. The ML trees were generated by performing a rapid bootstrap analysis in RAxML v.8.2.12 (Stamatakis, 2006; Stamatakis *et al*., 2008) with the GTR + I + G substitution model generated by jModeltest 2.1.6 (Darriba *et al*., 2012) and applying 1,000 bootstrap replicates. The BI analysis was performed on MrBayes 3.2.6 (Ronquist and Huelsenbeck, 2003) on the CIPRES using the best-fitting models that estimated separately each gene region (*matK*: GTR+G, ITS: GTR+I+G) (Miller *et al*., 2010). We ran the Markov chain Monte Carlo (MCMC) for 10 million generations, and trees were sampled every 1000 generations. Tracer v.1.4 was used to check the effective sample sizes (ESSs) of all relevant parameters (>200) (Rambaut and Drummond, 2007). The obtained trees were visualized using FigTree v.1.4.0 (Rambaut, 2009).

3. Results and Discussion

3.1. Molecular phylogenetic placement of Mokara Dear Heart: Congruence among nuclear and chloroplast phylogenetic analyses

The lengths of the two single data sets *mat*K and ITS were 1642 bps, and 710 bps, respectively. The combined dataset included 84 individuals with lengths of matrix 2352 bps. The topologies from two single data sets *mat*K, ITS, and the combined dataset from ML and BI analyses were highly congruent, and we thus presented the results from the three data sets (*mat*K, ITS, and the combined datasets) in the ML tree with bootstrap values from ML and BI analyses in Figs. 1. 2 and 3, respectively.

Our molecular data well supported that *Mokara* Dear Heartis placed in Vandeae by both single data sets (*mat*K and ITS) and combined dataset (Figs. 1, 2, 3). Our results indicate that the genus *Vanda* forms a clade containing some members of the genera *Ascocentrum*, *Christensonia*, and *Mokara* Dear Heart also seen in Zou *et al*. (2015), in spite of the fact that the species *Vanda malipoensis* is placedwith *Ascocentrum christensonianum* and *Ascocentrum pusillum* in a separate clade (Fig. 3).

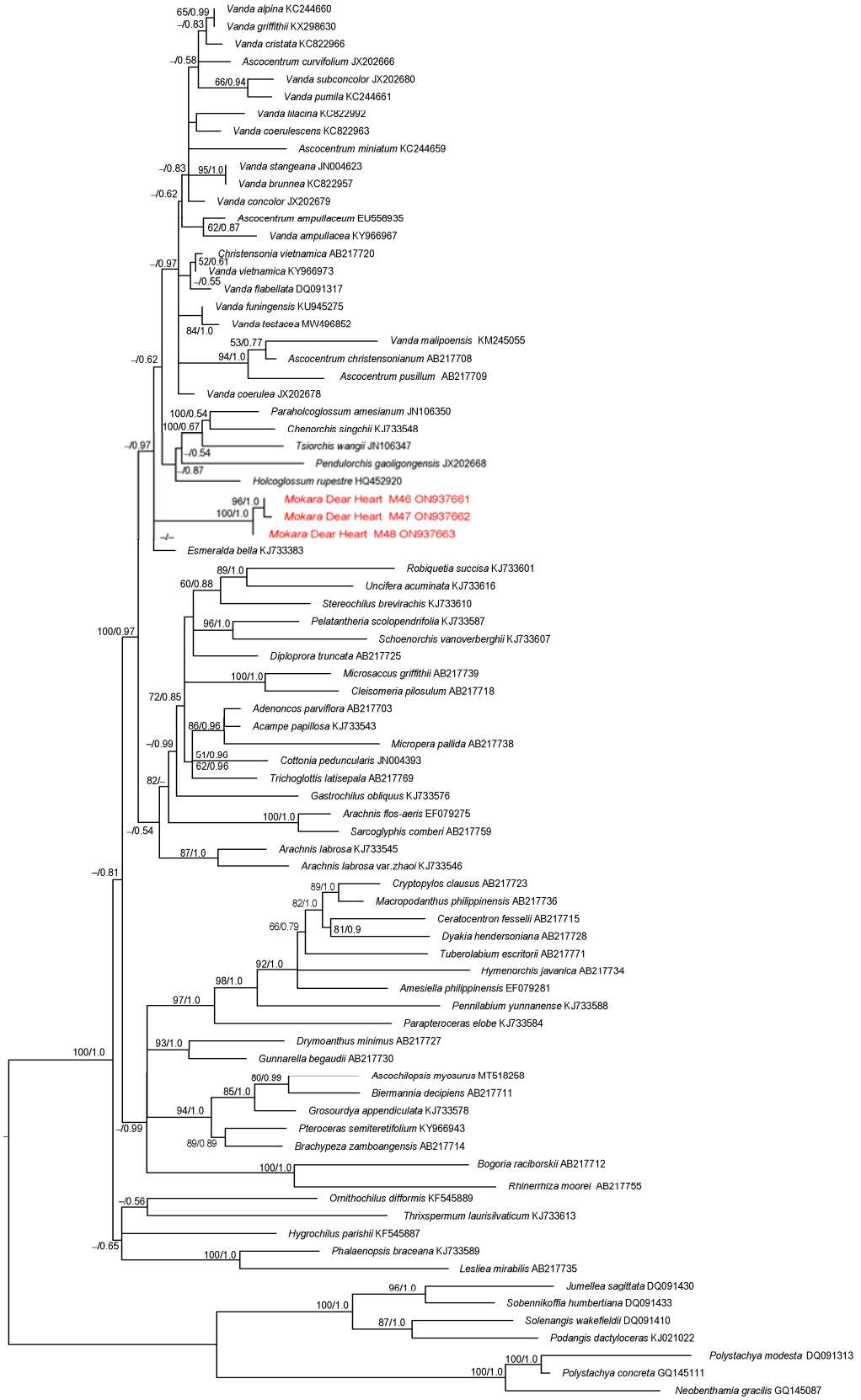

Figure 1. Maximum likelihood tree of *Mokara* Dear Heart based on the *matK* datasets. The bootstrap values from ML and BI analyses are displayed on the nodes. The symbol "–" denotes support values less than 50%.

Figure 2. Maximum likelihood tree of *Mokara* Dear Heart based on the ITS datasets. The bootstrap values from ML and BI analyses are displayed on the nodes. The symbol "–" denotes support values less than 50%.

The ITS sequences strongly supported *Mokara* Dear Heart placed within the *Vanda* genus. The three individuals of *Mokara* Dear Heart formed a clade with strongly supported (Bootstrap value (BS) = 100%; Posterior probability (PP) = 1.0). The genus *Ascocentrum* is non-monophyletic, two clades were recognized for *Ascocentrum*, and they are both placed within *Vanda* (Fig. 1). The first clade includes *A. ampullaceum*, *A. curvifolium*, *A. miniatum*, and *A. aurantiacum*, while another clade consists of *A. christensonianum* and *A. pusillum* formed as sister to *Vanda* with weakly supported (Fig. 2). Whereas, one of the three *Mokara*'s parent the genus *Arachnis* placed far from the *Vanda* clade. Thus, molecular analysis of ITS supported the non-monophyletic of *Vanda*, and *Mokara* Dear Heart is closely related to *Vanda*, while the *mat*K data indicated that *Mokara* Dear Heart formed as a sister to *Vanda* and *Ascocentrum*. However, the genus *Ascocentrum* is polyphyletic and placed within *Vanda* (Gardiner *et al*., 2013). The genus *Arachnis* was weakly placed far from the three genera (Fig. 2), and this genus was also recognized as non-monophyletic.

The results from the combined dataset strongly supported the placement of *Mokara* Dear Heart within the

Vanda genus (Fig. 3). Similarity is a situation of *Ascocentrum* and *Christensonia* genera. However, our results also indicated that *Ascocentrum* is non-monophyletic with *A. pusillum*, *A. christensonianum*, and *Vanda malipoensis* formed in a separate clade (Fig. 3). The nest of *Ascocentrum* within *Vanda* seems to result from the pollination of ancestors between the two genera through time and space.

Therefore, our molecular data supported that *Mokara* Dear Heart is closely related to its parent the genus *Vanda*, but it is far from *Arachnis*.

3.2. Genetic traits of Mokara suggested by molecular data

The *Mokara* is a Vandaceous member that is the result of a trigeneric cross between *Ascocentrum*, *Arachnis*, and *Vanda*. However, questions on the commercial and scientific names especially genetics of the hybrid lineages are needed to resolve. Based on the molecular results, we see that the similar position of *Mokara* in phylogenetic trees from nuclear (ITS) and chloroplast (*mat*K) data suggests that the genetics of the organelle plastid and nucleus of *Moraka* are linked (Figs. 1, 2), with the chloroplast a type of plastid and much of the genetic materials in the nucleus of *Moraka* derived from the *Vanda* lineage.

Figure 3. Maximum likelihood tree of *Mokara* Dear Heart based on the combined datasets from ITS and *matK*. The bootstrap values from ML and BI analyses are displayed on the nodes. The symbol "–" denotes support values less than 50%.

The molecular results supported that the hybrid orchid *Mokara* Dear Heart is a bigeneric hybridization and likely originated from *Arachnis* and *Vanda*. In which, *Arachnis* is the seed parent and *Vanda* is the pollen parent.

Indeed, angiosperms normally inherit both the chloroplast and mitochondrial genomes from their mothers (Camus *et al.*, 2022; Birky, 2008). However, for the hybrid orchid *Mokara* Dear Heart, we find that the chloroplast genome seems derived from the *Vanda* lineage (Fig. 1). Thus, this result is unexpected. Biparental inheritance of organellar genomes has been reported in several studies (Fauré *et al*., 1994; Testolin and Cipriani, 1997; Havey *et al*., 1998; Yang *et al*., 2000). Furthermore, in angiosperms, we find that several paternal inheritances of chloroplast genomes were discovered such as *Helianthus verticillatus* (Ellis *et al*., 2008) Celastrales–Oxalidales–Malpighiales (COM) clade (Sun*et al*., 2015), four Australian *Callitris* species (Cupressaceae) (Sakaguchi *et al*., 2012), and *Larrea* (Yang *et al*., 2000). Sun *et al.* (2015) mentioned the possibility of paternal chloroplast transmission to the COM clade's ancestor, but mitochondria are still maternally inherited. Moreover, the hybrid lineage may exhibit strong paternal chloroplast inheritance. For the *Mokara* Dear Heart case, in spite of the mitochondrial sequences being absent in previous and the current study, however, it is likely maternally inherited as other taxa in angiosperms (Fig. 4). Thus, it is possible that a complex hybridization event resulted in the chloroplast and mitochondrial genomes divergence of *Mokara* Dear Heart.

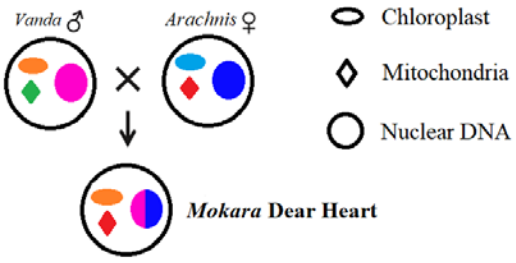

Figure 4. Mapping simulation of the hybrids orchid genus *Mokara* Dear Heart from *Arachnis* and *Vanda*. Plant lineages are represented by large circles, while nuclear DNA types are represented by small circles, chloroplasts are represented by ovals, and mitochondria are represented by diamonds. During the hybridization, the chloroplast genome is paternally inherited from the *Vanda*, while the mitochondrion genome is maternally inherited from the *Arachnis*.

In this hybridization scenario of *Mokara* Dear Heart, the *Vanda* Yip Sum Wah acted as the father parent and hybridized with *Arachnis* Maggie Oei's maternal lineage, resulting in paternal chloroplast transfer to the *Mokara* Dear Heart (Fig. 4). Thus, the hybridization event could be a reason for the conflict between the chloroplast and mitochondrial genomes, as well as competition among nuclear loci with half of the alleles in offspring contributed by each parent. That resulted in the close relationship between hybrid lineage and the paternal parent of Mokara based on chloroplast and nuclear data. Additionally, the commercial name *Mokara* Dear Heart should be corrected to *Aranda* Dear Heart.

4. Conclusions

This study represents a comprehensive phylogenetic relationship of the hybrids orchid genus *Mokara* Dear Heart. *Mokara* Dear Heart is closely related to its parent the genus *Vanda*, but far from *Arachnis*. The genetics of the organelles chloroplast and nucleus of *Moraka* are linked, with the chloroplast of *Mokara* Dear Heart apparently derived from the *Vanda* lineage. The hybrid orchid *Mokara* Dear Heart is a bigeneric hybridization originated from *Arachnis* and *Vanda*. In the hybridization scenario of *Mokara* Dear Heart, the *Vanda* Yip Sum Wah acted as the father parent and crossed with *Arachnis* Maggie Oei's maternal lineage, resulting in paternal transmission of the chloroplast to the *Mokara* Dear Heart. The commercial name *Mokara* Dear Heart should be corrected to *Aranda* Dear Heart.

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Author Contributions

C.T.L. P.B.C and V.D.N designed the study. V.C.H. collected the data. V.C.H., C.T.L. and P.B.C analyzed the data. All authors wrote and gave final approval for publication.

References

Birky CW. 2008. Uniparental inheritance of organelle genes. *Curr Biol*., **18**: R692–695.

Camus MF, Alexander-Lawrie B, Sharbrough J and Hurst GDD. 2022. Inheritance through the cytoplasm. *Heredity*, 1–13.

Carlsward BS, Whitten, WMark, Williams NH and Bytebier B. 2006. Molecular phylogenetics of Vandeae (Orchidaceae) and the evolution of leaflessness. *Am J Bot.*, **93**: 770–786.

Chen X, Liu Z, Zhu G, Lang K, Ji Z, Luo Y, Jin X, Cribb PJ, Wood JJ, Gale SW, Ormerod P, Vermeulen JJ, Wood HP, Clayton D and Bell A. 2009. Orchidaceae. In: Wu ZY, Raven PH and Hong DY (Eds.), **Flora of China**. Vol. 25. Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis.

Dalayap RM, Torres, MAJ and Demayo CG. 2011. Landmark and outline methods in describing petal, sepal and labellum shapes of the flower of mokara orchid varieties. *Int J Agric Biol.*, **13**: 652–658.

Darriba D, Taboada GL, Doallo R and Posada D. 2012. JModelTest 2: more models, new heuristics and high-performance computing. *Nature Methods*, **9**: 772.

Ellis JR, Bentley KE and McCauley DE. 2008. Detection of rare paternal chloroplast inheritance in controlled crosses of the endangered sunflower *Helianthus verticillatus*. *Heredity*, **100:** 574–580.

Farris JS, Källersjö M, Kluge AG and Bult C. 1995 Constructing a significance test for incongruence. *Syst Biol.*, **44:** 570–572.

Fauré S, Noyer JL, Carreel F, Horry JP, Bakry F and Lanaud C. 1994. Maternal inheritance of chloroplast genome and paternal inheritance of mitochondrial genome in bananas (*Musa acuminate*). *Curr Genet.*, **25**: 265–269.

Gardiner LM, Kocyan A, Motes M, Roberts DL and Emerson BC. 2013. Molecular phylogenetics of *Vanda* and related genera (Orchidaceae). *Bot J Linn Soc*, **173**: 549–572.

Havey MJ, McCreight JD, Rhodes B and Taurick G. 1998. Differential transmission of the *Cucumis organellar* genomes. *Theor Appl Genet.*, **97**: 122–128.

Kearse M, Moir R A, Wilson S, Stones-Havas M, Cheung S, Sturrock S, Buxton A, Cooper S, Markowitz C, Duran T, Thierer B, Ashton M and Drummond A. 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatic*, **28**: 1647–1649.

Lee YH. 1994. Genomic and meiotic analysis of *Mokara* orchids. *J. Hered*., **85(1):** 39–43.

Miller MA, Pfeiffer W and Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the gateway computing environments workshop (GCE). Pp. 1–8. New Orleans: Institute of Electrical and Electronics Engineers (IEEE).

Peyachoknagul S, Nettuwakul C, Phuekvilai P, Wannapinpong S and Srikulnath K. 2014. Development of microsatellite markers of vandaceous orchids for species and variety identification. *Genet Mol Res.*, **13**: 5441–5445.

Rambaut A. 2009. FigTree v.1.4. Available at: http://tree.bio.ed.ac.uk/software/figtree/

Rambaut A and Drummond AJ. 2007. Tracer. Version 1.4. Available at:http://beast.bio.ed.ac.uk/Tracer

Raskoti BB and Ale R. 2021. DNA barcoding of medicinal orchids in Asia. *Sci Rep.*, **11:** 23651.

Ronquist F. 1997. Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. *Syst Biol.*, **46**: 195–203.

Ronquist F and Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**: 1572–1574.

Ruhfel BR, Gitzendanner MA, Soltis DE, Soltis PS and Burleigh JG, 2014. From algae to angiosperms – inferring the phylogeny of green plants (Viridiplantae) from 360 plastid genomes. *BMC Evol Biol.*, **14**: 23.

Sakaguchi S, Tsumura Y, Crisp MD, Bowman DMJS and Isagi Y. 2014. Genetic evidence for paternal inheritance of the chloroplast in four Australian *Callitris* species (Cupressaceae). *J For Res.*, **19**: 244–248.

Soltis DE and Soltis PS. 2009. The role of hybridization in plant speciation. *Annu Rev Plant Biol.*, **60**: 561–588.

Soltis DE, Smith SA, Cellinese N, Wurdack KJ, Tank DC, Brockington SF, Refulio-Rodriguez NF, Walker JB, Moore MJ, Carlsward BS, Bell CD, Latvis M, Crawley S, Black C, Diouf D, Xi Z, Rushworth CA, Gitzendanner MA, Sytsma KJ, Qiu YL, Hilu KW, Davis CC, Sanderson MJ, Beaman RS, Olmstead RG, Judd WS, Donoghue MJ and Soltis PS. 2011. Angiosperm phylogeny: 17 genes, 640 taxa. *Am J Bot.*, **98**: 704–730.

Soon TE. 1989. Orchids of Asia. Times Book International, Times Center, Singapore.

Stamatakis A. 2006. RAxML-VI-HPC, maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, **22**: 2688–2690.

Stamatakis A, Hoover P and Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML Web servers. *Syst Biol.*, **57**: 758–771.

Sun M, Soltis DE, Soltis PS, Zhu X, Burleigh JG and Chen ZD. 2015. Deep phylogenetic incongruence in the angiosperm clade Rosidae. *Mol Phylogenet Evol.*, **83**: 156–166.

Szlachetko DL, Górniak M, Kolanowska M, Mytnik-Ejsmont J, Kowalkowska AK, Rutkowski P and Kolinski T. 2014. Taxonomic position and phylogeny of the genus *Vargasiella* (Orchidaceae, Vandoideae) based on molecular and morphological evidence. *PLoS ONE*, **9**: e98472.

Testolin R and Cipriani G. 1997. Paternal inheritance of chloroplast DNA and maternal inheritance of mitochondrial DNA in the genus *Actinidia*. *Theor App Genet*, **94**: 897–903.

Woo T D, Nakamoto W K. 1990. Do orchids grow in Hawaii? and how!. The Hawaii orchid foundation for the American orchid society, Hawaii.

Yang TW, Yang YA and Xiong Z. 2000. Paternal inheritance of chloroplast DNA in interspecific hybrids in the genus *Larrea* (Zygophyllaceae). *Am J Bot.*, **87**: 1452–1458.

Zhang GQ, Liu KW, Chen LJ, Xiao XJ, Zhai JW, Li Q, Cai J, Hsiao YY, Rao WH, Huang J, Ma XY, Chung SW, Huang LQ, Tsai WC and Liu ZJ. 2013. A new molecular phylogeny and a new genus, *Pendulorchis*, of the *Aerides–Vanda* Alliance (Orchidaceae: Epidendroideae). *PLoS ONE*, **8**: e60097.

Zou LH, Huang JX, Zhang GQ, Liu J and Zhuang XY. 2015. A molecular phylogeny of Aeridinae (Orchidaceae: Epidendroideae) inferred from multiple nuclear and chloroplast regions. *Mol Phylogenet Evol.*, **85**: 247–254.

Supporting Information

AppendixS1. GenBank accession numbers for DNA sequences generated or used in this study. The sequences generated in this study begin with ON. "–" indicates missing data.