A New Orchid Genus, *Danxiaorchis*, and Phylogenetic Analysis of the Tribe Calypsoeae

Jun-Wen Zhai1,2,3, Guo-Qiang Zhang2,8, Li-Jun Chen2, Xin-Ju Xiao2, Ke-Wei Liu2,7, Wen-Chieh Tsai4, Yu-Yun Hsiao4, Huai-Zhen Tian5, Jia-Qiang Zhu6, Mei-Na Wang2, Fa-Guo Wang1, Fu-Wu Xing1*, Zhong-Jian Liu2,7,8,9*

1 South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, China, 2 Shenzhen Key Laboratory for Orchid Conservation and Utilization, The National Orchid Conservation Center of China and The Orchid Conservation and Research Center of Shenzhen, Shenzhen, China, 3 Graduate University of Chinese Academy of Sciences, Beijing, China, 4 Institute of Tropical Plant Sciences and Orchid Research Center, National Cheng Kung University, Tainan City, Taiwan, 5 School of Life Science, East China Normal University, Shanghai, China, 6 Information Center of Renhua County, Shaoguan, China, 7 Center for Biotechnology and BioMedicine, Graduate School at Shenzhen, Tsinghua University, Shenzhen, China, 8 Landscape College of Fujian Agriculture and Forestry University, Fuzhou, China, 9 College of Forestry, South China Agricultural University, Guangzhou, China

Abstract

**Background:** Orchids have numerous species, and their speciation rates are presumed to be exceptionally high, suggesting that orchids are continuously and actively evolving. The wide diversity of orchids has attracted the interest of evolutionary biologists. In this study, a new orchid was discovered on Danxia Mountain in Guangdong, China. However, the phylogenetic clarification of this new orchid requires further molecular, morphological, and phytogeographic analyses.

**Methodology/Principal Findings:** A new orchid possesses a labellum with a large Y-shaped callus and two sacs at the base, and cylindrical, fleshy seeds, which make it distinct from all known orchid genera. Phylogenetic methods were applied to a matrix of morphological and molecular characters based on the fragments of the nuclear internal transcribed spacer, chloroplast matK, and rbcL genes of Orchidaceae (74 genera) and Calypsoeae (13 genera). The strict consensus Bayesian inference phylogram strongly supports the division of the Calypsoeae alliance (not including *Dactylolasia* and *Ephippianthus*) into seven clades with 11 genera. The sequence data of each species and the morphological characters of each genus were combined into a single dataset. The inferred Bayesian phylogram supports the division of the 13 genera of Calypsoeae into four clades with 13 subclades (genera). Based on the results of our phylogenetic analyses, Calypsoeae, under which the new orchid is classified, represents an independent lineage in the Epidendroideae subfamily.

**Conclusions:** Analyses of the combined datasets using Bayesian methods revealed strong evidence that Calypsoeae is a monophyletic tribe consisting of eight well-supported clades with 13 subclades (genera), which are all in agreement with the phytogeography of Calypsoeae. The Danxia orchid represents an independent lineage under the tribe Calypsoeae of the subfamily Epidendroideae. This lineage should be treated as a new genus, which we have named *Danxiaorchis*, that is parallel to *Yoania*. Both genera are placed under the subtribe Yoaniinae.

Citation: Zhai J-W, Zhang G-Q, Chen L-J, Xiao X-J, Liu K-W, et al. (2013) A New Orchid Genus, *Danxiaorchis*, and Phylogenetic Analysis of the Tribe Calypsoeae. PLoS ONE 8(4): e60371. doi:10.1371/journal.pone.0060371

Editor: Maria Anisimova, Swiss Federal Institute of Technology (ETH Zurich), Switzerland

Received November 25, 2012; Accepted February 12, 2013; Published April 4, 2013

Copyright: © 2013 Zhai et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: The work was supported by fellowships of the Forestry Construction of State Forestry Administration of China (No. 2010–240) and the Forestry Construction of Guangdong Province, China (No. 2010–317). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: xinfw@scib.ac.cn (FWX); liuzj@sinicaorchid.org (Z-JL)

Introduction

Orchidaceae is one of the largest families of angiosperms [1]. It has been said “The speciation rate in orchids is frequent because of their diverse flower and vegetable morphologies” [2]. Considerable attention has been given to their extraordinary pollination, multiple adaptive strategies to various habitats, and numerous dust-like seeds that lack endosperms [3,4]. Previously, Orchidaceae has been divided into five subfamilies based on their phytogeography [5]. Epidendroideae is a highly evolved and diverse subfamily, in which a few species are mycotrophic and lack green leaves. Many holomycotrophic orchids are found in China, comprising approximately 20 genera. None of these orchids has a bisaccate labellum.

Vanilla, *Apostasia*, *Cyrtosia*, *Palmorchis*, *Selenipedium*, and several *Neuwiedia* species have wingless seeds with hard seed coats. Several members of *Vanillea*, such as *Epipedium* and *Galeola*, have a hard seed coat over the embryo and a developed wing around the seed. Several *Neuwiedia* species have small seeds with sac-like appendages.
at either end. Most other orchids have a loose, rather papery seed coat around the embryo, which has a length that ranges from 0.15 mm to 6 mm. However, all of these orchid seeds are dry and lack an endosperm.

In this report, we documented a new orchid found on Danxia Mountain in Guangdong, China. The flower and seed structures of this new orchid are different from those of other known taxa in Orchidaceae. However, the phylogenetic clarification of this new orchid requires further molecular, morphological, and phytogeographic analyses.

Results

Morphological Analysis

The new orchid entity is restricted to the Danxia Mountain in northern Guangdong, China (Fig. S1). The Danxia region, known as the Danxia Landform, is famous for its topographic features. A detailed comparison between the newly discovered orchid and other members of Orchidaceae was conducted. The new plant is characterized by a labellum with two sacs at the base, an elongated column that has a terminal concave stigma and lacks conspicuous staminodes and rostellum, four sectile pollinia attached by two caudicles to a common large viscidium, and cylindrical, fleshy seeds. These features distinguish the new orchid from all other known orchids (Figs. 1, 2, and S2).

Analyses of Phylogenetic Placement

Danxiaorchis singhiana is morphologically related to the tribe Calypsoeae and, to a lesser degree, to the tribe Gastrodieae. Both of these tribes belong to the subfamily Epidendroidae. A detailed morphological character matrix (59 characters of 74 taxa) was integrated with a molecular matrix (3386 nucleotide sequences of the ITS, matK, and rbcL genes of 74 genera) to classify the plant into an appropriate phylogenetic position (Figs. 3, S3, S4, and S5).

Bayesian inference (BI) phylogram showed the monophyly of the new orchid plant. Five clades were distinguished in Orchidaceae, with a posterior probability (PP) of over 99% (Fig. 3). Based on evolutionary sequences, the five clades correspond to the subfamilies Apostasioideae, Cypripedioideae, Vanilloideae, Orchidoideae, and Epidendroidae. Calypsoeae formed an independent lineage (PP = 52%) in the subfamily Epidendroidae, under which Danxiaorchis is included together with other genera of Calypsoeae. In the family-level BI phylogram of the combined ITS, matK, and rbcL gene sequences, the Calypsoeae clade is divided into two subclades (PP = 95%, Fig. S4). The first subclade includes Calypso, Tipularella, and Changnienia, whereas the second subclade comprises eight genera, including Danxiaorchis, which is most closely related to Tipularella, as confirmed by maximum parsimony (MP) analysis (Fig. S5).

Phylogeny of Calypsoeae

Nuclear ITS sequence data analysis. The phylogenetic trees generated based on the ITS sequence data analysis clearly revealed the independence of the eight genera of this tribe. The BI phylogram with most of the clades received a strong support (PP>90%). Danxiaorchis, which forms a single clade with a PP of 99%, has been recognized as a natural genus within this tribe (Fig. S6). However, a relatively weak bootstrap and unstable topology is found in MP phylogram (Fig. S7).

Chloroplast sequence data analysis. Similarly, the 11 genera can be easily distinguished from the phylograms based on chloroplast sequence data analysis. The phylogenetic topologies generated by BI are approximately congruent with the ones by MP analysis (Figs. S8 and S9). The basal clade is independently composed of Calypso, Tipularella, and Changnienia. The next clade is Govenia, followed by a complex clade, which includes Aplectrum, Cremastra, Danxiaorchis, Yoania, Wallisichangnienia, and Oreorchis. Corallorhiza occupies the terminal positions in both MP and BI phylograms, although it is not well-supported intragenetically in the MP phylogram.

Combined analysis. In this study, ITS, matK, and rbcL were combined into a single dataset. The strict consensus BI phylogram (Fig. S10) strongly supports the division of the Calypsoeae alliance (except Dactylostalix and Ephippianthus) into seven clades with eight subclades (PP = 100%, except for one with 77%). The first clade, which consists of the Changnienia, Tipularella, and Calypso subclades, is strongly supported as a sister to the outgroup clade, which consists of Sobralia and Nervilia (PP = 100%). The second clade, which has a single genus, Govenia, is strongly supported as a sister to the first clade (PP = 100%). The third clade is the Aplectrum genus, and the fourth is Cremastra (PP = 100%). The fifth clade contains the new genus Danxiaorchis, and its ally, Yoania (PP = 100%). The last two clades have weak support (PP = 77%). The sixth clade is comprised of Wallisichangnienia and Oreorchis. The seventh clade contains a single genus, Corallorhiza, which consists of 13 species that are further divided into two subclades. The results are in agreement with the results of the complex clades in the MP phylogram (Fig. S11).

The sequence data of each species and the morphological characters of each genus were combined into a single dataset (Dactylostalix and Ephippianthus having morphological characters only). The strict consensus BI phylogram supports the division of the 13 genera of Calypsoeae into four clades with 13 subclades, which is in agreement with the results of the combined sequence data analysis. These results show that the genera Dactylostalix and Ephippianthus belong to a single clade near the Govenia and Calypso clades (Figs. 4 and 5).

Discussion

Morphological Analysis

This study is the first to report an orchid with a bisaccate labellum and cylindrical, fleshy seeds. This orchid is difficult to classify in any known subtribe or tribe within Orchidaceae. Although the new orchid has a particular similarity to Gastrodia and its allies in terms of pollinium structure, the pollinarium of Gastrodia lacks distinct caudicles and viscidium like the new orchid does. This new orchid sharply differs from Satyrium and Corybas except for the two elongate or saccate spurs at the base of their labellum [9]. Danxiaorchis has cylindrical (1.5 mm×0.5 mm) and fleshy seeds, which is similar to the seeds of underground orchid species of Rhizanthella [10]. However they’re distinct from Danxiaorchis by its underground habitat, absence of roots, fleshy overlapping bracts, small flowers and minute seeds. Thus, this genus is distinguishable from all other genera of orchids.

Family-level Analysis

The results of our analyses are in agreement with those obtained by previous researchers [5,9,11,12] and support the relationships among the subfamilies Apostasioideae, Vanilloideae, Cypripedioideae, Orchidoideae, and Epidendroidae. In addition, a more precise phylogenetic tree was obtained in the present study at higher categories. This finding may be attributed in part to the utilization of a more diversified nuclear genetic marker, ITS, the application of multiple genetic markers, and the integration of morphological and molecular characters.
Figure 1. *Danxiaorchis singchiana* flowers and seeds. (A) Flowers with a Y-shaped appendage (arrow) on the labellum. Bar = 1 cm. (B) Labellum with two sacs (arrows) at the base. Bar = 4 mm. (C) Column and labellum, side view. Bar = 4 mm. (D) Appendage of the labellum, side view. Bar = 2 mm. (E) Pollinarium, front view, showing pollinia (red arrows), caudicles (white arrows), and viscidium (yellow arrow). Bar = 1 mm. (F) Mature seeds, showing abortive seed (yellow arrow). Bar = 5 mm. doi:10.1371/journal.pone.0060371.g001
Calypsoeae-level Analysis

Dressler [5] and Pridgeon et al. [ed.] [13] investigated the tribe Calypsoeae as comprising nine genera, namely, Aplectrum, Calypso, Corallorhiza, Cremastra, Dactylostalix, Ephippianthus, Oreorchis, Tipularia, and Yoania. Pridgeon et al. [ed.] [13] added Wullschlagelia and Govenia to this tribe, although Dressler had placed the former in Gastrodiinae and the latter in Cymbidieae [5], both at subtribal rank. Meanwhile, Chen et al. [14] treated these genera as members of the tribe Epidendreae, with the following subtribes in China: subtribe Yoaniinae with the genus Yoania; subtribe Calypsoinae with the genera Oreorchis, Cremastra, Tipularia, Calypso, and Changnienna; and subtribe Corallorhizinae with the genus Corallorhiza. However, all of these classification systems were based only on morphological evidence.

Govenia and Corallorhiza are both monophyletic genera (PP = 100%), with the former genus having a relatively anomalous distribution in the tribe Calypsoeae [13]. Thus, their subtribal rank, the subtribes Goveniinae [5] and Corallorhizinae [14], is maintained. The phylogenetic placement and the infrageneric relationships of Corallorhizinae are very similar to those studied by Freudenstein et al. [15]. Corallorhiza should be divided into two subclades. The first subclade contains C. strata Lindl., C. bentleyi Freudenst., C. involuta Grem., and C. veitchii Rydb. The species of this subclade possess three-veined perianth segments and a thickened labellum that are fused at the base. The second subclade contains the rest of the species in this genus. They possess a thin-textured labellum, and some species, such as C. trifida Châtel, and C. odontorhiza (Willd.) Nutt. are autogamous [15–18].

Aplectrum and Wullschlagelia are composed of a few species that are distributed from North America to tropical South America. For Wullschlagelia, a monotypic subtribe Wullschlaegelinae was established in 1990 [5]. Cremastra and Oreorchis are only found in Asia [19,20]. Yoania is most closely related to Danxiaorchis and they share the same habitat. However, Danxiaorchis can be distinguished from Yoania by its rooted rhizome, bisaccate labellum, Y-shaped appendages, and caudicles [21].

The Danxia Mountain located in northern Guangdong, where Danxiaorchis grows, was formed approximately 6 million years ago [22]. The unique geological conditions and the relative isolation of the Danxia Mountain might have favored the speciation of new taxa, such as Oberonia taxanthera (Schltr.) Szlach. [14], Firmiana danxiaensis H. H. Hsue et H. S. Kiu [23], and Lyonia danxiaensis Miu et W. Q. Liu [24].

Two distantly related genera, Dactylostalix and Ephippianthus [13], are distributed along the Sakhalin Peninsula in northern Japan and the Kuriles. Unfortunately, materials from these two genera could not be obtained. However, in our study, these two genera formed a sister clade with the Calypso and Govenia clades based on their morphological characters.

Conclusion

The Danxia orchid has several distinct features. Based on results obtained by applying phylogenetic methods to a matrix of morphological and molecular characters, the Danxia orchid can be treated as a new genus of Calypsoeae (subfamily Epidendroideae). Analysis of the combined datasets using maximum likelihood methods revealed strong evidence that Calypsoeae is a monophyletic tribe consisting of eight well-supported clades with 13 subclades, which are all in agreement with the phytogeography of Calypsoeae.

The Danxia orchid represents an independent lineage under the tribe Calypsoeae of the subfamily Epidendroideae. This lineage should be treated as a new genus parallel to Yoania under the subtribe Yoaniinae. The new classification should be as follows: Subfamily: Epidendroideae

Tribe: Calypsoeae

Subtribe: Yoaniinae Szlach.

Danxiaorchis singchiana J. W. Zhai, F. W. Xing, and Z. J. Liu gen. et sp. nov. (Figs. 1, 2, and S2) [Danxiaorchis, urn:lsid:ipni.org:names: 77124909-1; D. singchiana, urn:lsid:ipni.org:names: 77124909-1].

Etymology: The generic name alludes to Danxia, the name of the locality where it was found. The Danxia Mountain is famous for its topographic feature, the Danxia landform. The Greek name for orchid, orchis, is then incorporated. Thus,
Figure 3. Bayesian consensus phylograms for the combined ITS, matK, and rbcL datasets and 59 morphological character matrix, including 72 genera of Orchidaceae. The Bayesian PP (×100) is provided above the branches.
doi:10.1371/journal.pone.0060371.g003
Danxiaorchis refers to an orchid growing on the Danxia Mountain. The specific epithet singchiana is dedicated to the Chinese professor Sing-Chi Chen, a famous, internationally renowned orchidologist.

- **Type**: Guangdong, Renhua, Danxianshan, in a forest, alt. 125 m, 2011.5.31. J. W. Zhai, 5481 (holotype, NOCC; isotype: IBSC).

- **Diagnosis**: The new remarkable genus is distinct from all known orchid genera by it possesses a labellum with a large Y-shaped callus and two sacs at the base, and cylindrical, fleshy seeds.

- **Description**: Holomycotrophic plants 21 to 40 cm tall; rhizome tuberous, fleshy, cylindrical, 5 to 6 cm long, 0.6 to 1.8 cm thick, shortly branched, rooting. Scape erect, terete, pale red-brown, slightly tinged with green-yellow, 3- to 4-sheathed; sheaths, cylindrical, clasping stem, membranous, 2.3 to 3.5 cm long; raceme 5 to 8.5 cm long, 2- to 13-flowered; floral bracts oblong-lanceolate, 1.5 to 2.3 cm long; pedicel and ovary 2.2 to 4.6 cm long, glabrous; sepals and petals pale yellow; lip yellow, with pale-purple-red stripes on side-lobes and purple-red spots on mid-lobe; dorsal sepal narrowly elliptic, 1.8 to 2.6 cm × 6 to 9 mm, acute; lateral sepals obovate-elliptic, 2 to 2.3 cm × 7 to 9 mm, acute; petals narrowly elliptic, 2 to 2.2 cm × 6.5 to 7.5 mm, acute; labellum 3-lobed; side-lobes erect, slightly clasping the column, subquadrate, up to 5 mm long and 5.5 mm wide; mid-lobe oblong, 7 to 8 mm × 5 to 8 mm, apex rounded-obuse; labellum with two sacs at the base and a Y-shaped fleshy appendage centrally; appendage extending from the base of the disc to the base of mid-lobe, 1.3 to 1.5 mm tall; column semi-seriate, 5 to 7 mm long, footless; stigma concave, terminal; anther cap elliptoid; pollinia four, in two pairs, subovoid-globose, granular-farinaceous, composed of friable massulae, each pair containing two pollinia unequal in size with the thick caudicle attached to a common subsquare viscidium. Capsule fusiform, 3 to 4.2 cm long, 0.8 to 1.2 cm thick. Seeds cylindrical, 1.5 × 0.5 mm, fleshy. Fl. April–May. Fr. May–June.

Materials and Methods

**Materials**

The locations of the field studies are neither private lands nor protected areas, but are controlled by the State Forestry Administration of China, to which our institution is affiliated. The State Forestry Administration authorized us to conduct scientific observations or tests in the regions it controls. A valid permit was also obtained for testing the genes of Danxiaorchis.

A total of 74 genera were analyzed in the family-level study. Two genera, Hypoxis and Carvalloare, were selected as outgroups. Three genetic markers (ITS, matK, and rbcL) of Danxiaorchis, Corallorhiza, Cremastea, Oreorchis, and Yoania were analyzed. The gene sequences of the other 61 genera were accessed from GenBank (Table S1). Danxiaorchis singchiana was collected from the Danxia Mountain in northern Guangdong, China (25°N, 113°E).

A total of 34 species (or subspecies or varieties) and 35 individuals of 13 genera were included in the tribe-level analysis, wherein Sobralia and Novelia were selected as outgroups. The ITS, matK, and rbcL gene sequences of Danxiaorchis singchiana, Corallorhiza trifida, Changnienia malipoensis, Cremascha appendiculata, Yoonia japonica, Oreorchis indica, and O. nana were applied in the same way as in the family-level study. The other sequences were accessed from GenBank (Table S2).

**Methods**

**Amplification and sequencing.** Total DNA was extracted from fresh material, silica gel-dried plant tissue, or herbarium specimens using a modified hexadecyl trimethyl ammonium bromide method [25].

The amplification reaction included total DNA, primers, Mighty Amp buffer version 2.0, and Mighty Amp DNA polymerase (Takara Bio). The polymerase chain reaction (PCR) profile consisted of an initial 2 min pre-melt stage at 98°C; 35 cycles of 20 s at 98°C (denaturation), 20 s at 55°C to 55°C (annealing temperature was determined by the requirements of the primer), and 50 s to 90 s at 68°C (extension time was determined by the length of the target DNA region); and a final extension of 6 min to 8 min at 68°C. Amplification of the ITS, matK, and rbcL regions was separately performed using the primer pairs ITS A and ITS B, matK-19F and tmK-2R, and rbcL [26–28]. Other matK and rbcL primer sets were also amplified (Table S3). The PCR products were run on 1.5% agarose gels to check the amplified DNA quality. Gels with target products were excised, purified using DNA gel extraction kits (OMEGA BIO-TEK, USA), and sequenced by Invitrogen (Shanghai).

**Sequence editing and assembling.** The forward and reverse sequences as well as electropherograms were edited and assembled using DNASTAR (http://www.dnastar.com/). The DNA sequences were aligned using MEGAS5.05 using Muscle method [29] and then manually adjustments were made for inserting gaps to improve the alignments [30]. The aligned sequences are available from the corresponding authors upon request.

**Morphological analyses.** A matrix, which consists of 59 morphological characters of 74 taxa in the family-level analysis (Morphological Character Codes S1 and Table S4) and 69 morphological characters of 35 taxa in the tribe-level analysis (Table S3), was constructed to explore the phylogenetic positions of the Danxiaorchis alliance by morphological classification.

**Data analyses.** Maximum Parsimony (MP) analyses were performed using PAUP* version 4.0b10 [31]. All characters were equally weighted and unordered. The test settings included 1,000
replications of random addition sequence and heuristic search with tree bisection and reconnection branch swapping. Tree length, consistency indices (CI), and retention indices (RI) are shown in Table S6. BI analysis was performed using MrBayes3.1.2 [32]. The best-fit model for each dataset was selected using Modeltest 3.7. The model for the combined ITS, matK, and rbcL datasets was also based on the best-fit model for each individual dataset (Tables S7 and S8). The following settings were applied: sampling frequency = 100; temp = 0.1; burn-in = 10,000; and the number of Markov Chain Monte Carlo generations = 4,000,000. The first 10,000 trees were discarded as burn-in. A majority-rule consensus phylogram was constructed based on the phylograms sampled after the 1,000,000th generation.

Nomenclature Acts
The electronic version of this article in Portable Document Format (PDF) in a work with an ISSN or ISBN will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants, and hence the new names contained in the electronic publication of a PLOS ONE article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

In addition, new names contained in this work have been submitted to IPNI, from where they will be made available to the Global Names Index. The IPNI LSIDs can be resolved and the associated information viewed through any standard web browser by appending the LSID contained in this publication to the prefix http://ipni.org/. The online version of this work is archived and available from the following digital repositories: PubMed Central, LOCKSS.

Supporting Information

Figure S1  *Danxiaorchis* location. Map showing the *Danxiaorchis* locality (star) in the Danxia Landform in northern Guangdong Province, China. The inset map shows the location of Guangdong Province in southern China. (TIF)

Figure S2  *Danxiaorchis singchiana*. (A) Flowering plants in their habitat. Bar = 4 cm; (B) Inflorescence. Bar = 1.5 cm; (C) Fruiting plant. Bar = 2 cm; (D) Tuberous rhizome. Bar = 6 mm. (TIF)

Figure S3  Strict consensus phylogram of most parsimonious phylogenies based on the combined ITS, matK, and rbcL datasets and a matrix composed of 59 morphological characters of 72 *Orchidaceae* genera. Bootstrap values of the MP analysis are indicated above the branches. Tree length = 18095 steps; CI = 0.2354; RI = 0.5600. (TIF)

Figure S4  Bayesian consensus phylogram for the combined ITS, matK, and rbcL datasets, including 71 genera of *Orchidaceae*. Bayesian PP (×100) is indicated above the branches. (TIF)

Figure S5  Strict consensus phylogram of most parsimonious phylogenies based on the combined ITS, matK, and rbcL datasets, including 71 genera of *Orchidaceae*. Bootstrap values for the MP analysis are indicated above the branches. Tree length = 10188 steps; CI = 0.3248; RI = 0.5857. (TIF)

Table S1  Samples used in *Orchidaceae* gene sequencing and their information. (DOC)

Table S2  Samples used in *Calypsoeae* gene sequencing and their information. (DOC)

Table S3  Primers used in this study. (DOC)

Table S4  Morphological data matrix for the phylogenetic analysis. (DOC)

Table S5  Morphological data matrix for the tribe-level phylogenetic analysis. (DOC)

Table S6  Statistics from the analyses of various datasets. (DOC)
Table S7 Best-fit model and parameter for each Orchidaceae dataset.

Table S8 Best-fit model and parameter for each Calypsoeae dataset.

Morphological Character Codes S1.

Acknowledgments

We would like to thank Xu-Hui Chen, Wei-Rong Liu, and Wen-Hui Rao for their help in the field work; Xin-Lan Xu, Lin Fu, and Ru-Fang Deng for their help with Laser Scanning Confocal Microscopy; and Yu-Yun Zheng for helping in the manuscript preparation.

Author Contributions

Conducted taxonomic treatment: JWZ FWX ZJL LGQ GQZ. Conceived and designed the experiments: ZJL JWZ FWX. Performed the experiments: JWZ LGQ GQZ ZJL HZT GQZ FGW. Analyzed the data: JWZ GQZ YXH KWL ZJL FWX WCT LGQ. Contributed reagents/materials/analysis tools: JWZ ZJL FQZ GQZ LGQ XJX MNW. Wrote the paper: JWZ ZJL.

References

1. Govaerts RHA (2012) World checklist of selected plant families. Royal Botanic Gardens, Kew. Available: http://www.kew.org/science-research-data/directory/teams/monocots-iii/orchids/index.htm. Accessed 28 February 2013.
2. Gill DE (1989) Fruiting, Pollinator inefficiency, and speciation in orchid. In: Otto D, Endler JA, eds. Speciation and Its Consequences. Sinauer Associates Sunderland, 438–481.
3. Liu KW, Liu ZJ, Huang LQ, Li LQ, Chen LJ, et al. (2006) Self-fertilization strategy in an orchid. Nature, 441: 945–946.
4. Cozzolino S, Widmer A (2005) Orchid diversity: an evolutionary consequence of deception? Trends Ecol. Evol., 20: 487–496.
5. Dressler RL (1993) Phylogeny and Classification of the Orchid Family. Cambridge University Press, Cambridge.
6. Neubig KM, Whitten WM, Carlsward BS, Blanco MA, Endara L, et al. (2009) Phylogenetic utility of ycf1 in orchids: a plastid gene more variable than matK. Plant Syst. Evol., 277: 75–84.
7. Cameron KM, Chase MW, Whitten WM, Korres PJ, Jarrell DC, et al. (1999) A phylogenetic analysis of the Orchidaceae: evidence from rDNA nucleotide sequences. Am. J Bot., 86: 208–224.
8. Chase MW, Cameron KM, Hills H, Jarrell D (1994) DNA sequences and phylogenetics of the Orchidaceae and other lilioid monocots. In: Pridgeon A, ed. Proceedings of the Fourteenth World Orchid Conference. Her Majesty’s Stationery Office, Glasgow, UK. 61–73.
9. Chen SC, Liu ZJ, Zhou GH, Lang KY, Ji ZH, et al. (2009) Orchidaceae. In Wu ZY, Raven PH, Hong D, eds. Flora of China, vol. 25. Science Press, Beijing & Missouri Botanical Garden Press, St. Louis. 1–9.
10. Bougoure JJ, Bundrett MC, Grierson PF (2010) Carbon and nitrogen supply to the underground orchid, Rhizanthella gardneri. New Phytol., 186: 947–956.
11. Gorniak M, Paun O, Chase MW (2010) Phylogenetic relationships within Orchidaceae based on a low-copy nuclear coding gene, Xdh: Congruence with regular and nuclear ribosomal DNA results. Mol. Phylogenet. Evol., 36: 784–795.
12. Cameron KM (2004) Utility of plastid petB gene sequences for investigating intrafamilial relationships within Orchidaceae. Mol. Phylogenet. Evol., 31: 1157–1180.
13. Pridgeon AM, Cribb PJ, Chase MW, Rasmussen FN (2005) Genera Orchidacearum: Epidendroideae (part one), vol. 4: 89–115. Oxford University Press Inc., New York.
14. Chen SC, Tsi ZH, Lang KY, Zhu GH (1999) Flora Reipublicae Popularis Sinicae 18. Science Press, Beijing.
15. Freudenstein JV, Senyo DM (2008) Relationships and evolution of matK in a group of leafless orchids (Corallorhiza and Corallorhizinae; Orchidaceae: Epidendroideae). Am. J Bot., 95 (4): 498–505.
16. Freudenstein JV (1999) A new species of Corallorhiza (Orchidaceae) from West Virginia, U.S.A. Novon, 4: 511–513.
17. Claessens J, Kleynen J (2005) Pollination in the European orchids: four examples. 18th World Orchid Conference, Dijon, France.
18. Sun HQ, Luo YB, Alexander Freudenstein JV, ed. (2006) Pollination biology of the deceptive orchid Ganga anura anansa. Bot. J Linn. Soc., 150: 165–175.
19. Lund ID (1988) The genus Crematraga (Orchidaceae), a taxonomic revision. Nord. J Bot., 8: 197–203.
20. Pearce N, Cribb P (1997) A revision of the genus Oreorchis (Orchidaceae). Edinb. J Bot., 54 (3): 289–328.
21. Freudenstein JV (1994) Gymnostemium structure and relationships of the Corallorhizinae (Orchidaceae: Epidendroideae). Plant Syst. Evol., 193: 1–19.
22. Huang J (1999) On the distribution of Danxia landforms in China. Economic Geography, 19 (suppl.): 31–35.
23. Hsu HH, Kiu HS, Xu SJ (1987) New species and variety of Sterculia from China. Journal of South China Agriculture University, 8 (3): 1–5.
24. Liu WQ, Li ZH, Liu LF (1999) A preliminary study on the flora of the tourist landform of Danxiashan, Guangdong, China. Guihaia, 19(1): 15–21.
25. Mike T, Lena S, Joachim WK (1999) The phylogenetic relationships and evolution of the Canarian laurel forest endemic Ixanthusviscosus (Aiton) Griseb. (Gentianaceae): evidence from matK and ITS sequences, and floral morphology and anatomy. Plant Syst. Evol., 218: 299–317.
26. Reeves G, Chase MW, Goldblatt P, Rudall P, Fay MF, et al. (2001) Molecular systematics of Iridaceae: evidence from four plastid DNA regions. Am. J Bot., 88(11): 2074–2087.
27. Sulaiman SF, Culham A, Harborne JB (2003) Molecular phylogeny of Fabaceae based on matK and ITS sequences, and floral morphology. Plant Syst. Evol., 281: 1792–1797.
28. Edgar RC (2004) Muscle: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res., Vol. 32, No. 5, 1792–1797.
29. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, et al. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolution ary distance, and Maximum Parsimony methods. Mol. Biol. Evol., DOI: 10.1093/molbev/mes112.
30. Wu ZJ, Xu JW, Fu L, Deng RF, et al. (2008) A New Orchid Genus of Tribe Calypsoeae for their help with Laser Scanning Confocal Microscopy; and Yu-Yun Zheng for helping in the manuscript preparation.