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Goodyera medogensis (Orchidaceae), a new species from Tibet, China

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Abstract

A new species of *Goodyera* (Orchidaceae) from Tibet, China, *G. medogensis*, is described and illustrated here. This new species is most morphologically similar to *G. hemsleyana*, but differs by the thick grid lines of reticulations with blurred margin on adaxial surface of leaf blades, inflorescence with more flowers, petals obliquely obovate-rhombic, labellum yellow or yellowish, without lamella on blade, and floral bracts, sepals and ovary with non-glandular trichomes. Molecular phylogenetic study also supports that *G. medogensis* is a new species and belongs to *G.* subsection *Reticulum*.

Key words

Jewel orchid, morphology, new species, phylogeny

Introduction

The genus *Goodyera* R. Br. (Orchidaceae), known as jewel orchids, consists of about 100 species distributed in South Africa, Madagascar, Asia, Southwest Pacific islands, Northeast Australia, Europe, and North America (Chen et al. 2009). This genus belongs to the subtribe Goodyerinae of the tribe Cranichideae in the subfamily Orchidoideae (Pridgeon et al. 2003). *Goodyera* is characterized by elongate creeping rhizome, cymbiform lip with a concave-saccate hypochile, sectile pollinia and one stigma.

There are approximately 34 species of *Goodyera* in China, out of which 15 are endemic (Chen et al. 2009; Jin and Yang 2015; Zhou et al. 2016; Wang et al. 2020; Zhou et al. 2020). During a botanical survey in Medog County, Tibet Autonomous Region in January 2021, a few living plants of an unknown jewel orchid with reticulated leaves was collected and cultivated in Kunming Botanical Garden. It blooms vigorously with many beautiful flowers in August. We also found flowering individuals in the field at the same time. It is similar to *G. hemsleyana* King & Pantl. However, it has greenish flowers with yellow lips, while *G. hemsleyana* has pink flowers with white lips. Besides, the reticulations on leaf blades of the two species are also different.

Based on detailed examination of the morphological characters of our materials and its

similar species, and molecular phylogenetic evidence, we concluded that this species is new to science. It is described and illustrated here as *G. medogensis* H. Z. Tian, Y. H. Tong & B. M. Wang.

Materials and methods

Voucher specimens of *G. medogensis* were collected from Medog County and preserved at the herbarium of South China Botanical Garden, Chinese Academy of Sciences (IBSC) and East China Normal University (HSNU). Fresh leaves using for molecular analyses were preserved in silica gel. Descriptions were based on living materials. Measurements were performed with a ruler, and small plant parts were observed and measured under a stereo microscope (Mshot-MZ101).

To study the phylogenetic position of the new species within the genus *Goodyera*, three DNA fragments, viz. internal transcribed spacer (ITS) and two plastid DNA regions (*matK* and *trnL-F*), were selected for building the phylogenetic tree based on previous studies (Hu et al. 2016; Zhou et al. 2020). In total, 32 species represented by 76 samples of *Goodyera* were analyzed with one sample of *Pachyplectron arifolium* Schltr. as the outgroup. All sequences were downloaded from GenBank except for the new species. Species names and GenBank accession numbers are provided in supplementary materials Table S1.

Total genomic DNA of the new species was extracted from silica gel-dried leaves using a modified CTAB method (Doyle and Doyle 1987). Polymerase Chain Reaction (PCR) amplification was carried out on TAKARA TP600 thermocycler (TAKARA BIO INC, Japan) using 25 μ l reactions containing 12.5 μ l 2× Taq PCR Master Mix (HuaGene, China), 8.5 μ l ddH₂O, 1.5 μ l of each primer (10 μ M) and 1 μ l target DNA template. Detailed information of primers of relevant DNA fragments used in PCR amplification and sequencing, as well as the procedures of PCR, can be found in Table S2. The resulting amplicons were visualized by horizontal agarose gel electrophoresis (1%), colored with GoldView I (Solarbio, China). Clearly distinguishable bands were recorded, and then the corresponding PCR products were sequenced by Shanghai HuaGene Biotech Co., Ltd (Shanghai, China).

Sequences were firstly assembled and edited with Seqman (DNA STAR package, Madison, WI, USA) and then adjusted manually. Phylogenetic analysis was conducted using phylosuite ver. 1.2.2 (Zhang et al. 2020). Sequences were aligned with MAFFT (Katoh and Standley 2013). Ambiguously aligned fragments were removed using Gblocks (Talavera and Castresana 2007) with all parameters at their default settings. After that, we replace the "-" at both ends of the sequence with "?" instead to represent missing data using MEGA version X 10.1.6 (Kumar et al. 2018). Next, *matK* and *trnL-F* were concatenated as well as ITS, *matK* and *trnL-F* respectively to two datasets. Thus, three datasets were constructed in total: the cpDNA dataset (including *matK* and *trnL-F*). The three datasets were analyzed by using Bayesian inference (BI) and maximum likelihood (ML) methods respectively. Best-fit evolutionary model for Mrbayes and IQ-TREE were selected under the Bayesian Information Criterion (BIC) using ModelFinder (Kalyaanamoorthy et al., 2017). The best-fit models for the Maximum likelihood (ML) analysis are K80+R2 (ITS) and K3Pu+F+R2 (cpDNA,

nr+cpDNA), and for Bayesian inference (BI) they are K2P+G4 (ITS) and GTR+F+G4 (cpDNA, nr+cpDNA).

Based on these models, the Maximum Likelihood (ML) analysis was performed with IQ-TREE (Nguyen et al. 2015) for 10000 ultrafast (Minh et al., 2013) bootstraps, and Bayesian Inference (BI) phylogenies were inferred using MrBayes 3.2.6 (Ronquist et al. 2012). BI analysis consisted of two simultaneous runs and four simultaneous Markov Chain Monte Carlo (MCMC) chains, and ran for 3000000 generations with chain sampling every 1000 generations. The average deviation of split frequencies fell below 0.01, and initial 25% of sampled data were discarded as burn-in. The phylogenetic trees were visualized and modified in FigTree version 1.4.3 (Rambaut and Drummond 2016).

Results

Our ML and BI phylogenetic trees constructed by the three datasets showed that our four samples of *G. medogensis* cluster into one separate subclade (Figures 1a, 1b, 2), which locates in the clade of *Goodyera* subsection *Reticulum* S. W. Chung & C. H. Ou (Hu et al., 2016) consisting of other seven species, viz. *G. biflora* (Lindl.) Hook. f., *G. hachijoensis* Yatabe, *G. hemsleyana* King & Pantling, *G. hispida* Lindl., *G. pusilla* Bl., *G. vittata* Benth. ex Hook. and *G. yamiana* Fukuy., which have relatively obvious reticulations on the leaves and lip sac with hairs inside. Thus, the results of phylogenetic analyses supported that *G. medogensis* is a new species belonging to the subsection *Reticulum*, and it has close relationship with *G. biflora*, *G. hemsleyana* and *G. vittata*.

Taxonomic treatment

Goodyera medogensis H. Z. Tian, Y. H. Tong & B. M. Wang, sp. nov.

Figure 3

Type. China. Tibet Autonomous Region: Medog County, Renqingbeng Temple, under evergreen broad-leaved forest, cultivated in Kunming Botanical Garden, 3 August 2021 (fl.), *B. M. Wang TYH-2523* (holotype: IBSC, isotype: HSNU).

Diagnosis. Similar to *G. hemsleyana*, but distinguished by the grid lines of reticulations on adaxial surface of leaf blades thick with blurred margin (vs. thin with clear margin), inflorescence with more flowers ((6-)12-15 vs. 4-10), petals obliquely obovate-rhombic (vs. obliquely ovate-oblong), labellum yellow or yellowish (vs. white, with light greenish to pinkish tinge at apex) without lamella on blade (vs. with a low bi-lamellate callus), and floral bracts, sepals as well as ovary with non-glandular trichomes (vs. glandular).

Description. Terrestrial herb, 12–25 cm tall. Rhizome 4–6 cm long, 1.5–3 mm in diam., greenish, rooting at nodes. Roots fleshy, 0.7–7 cm long, yellowish brown, with minute root hairs. Stem erect, terete, 4–9 cm long, 2.7–4.0 mm in diam., pale green, glabrous, with few sheaths at base formed by withered bases of petioles. Leaves 3–7; petiole 1.1–1.6 cm long, sheathing at base; lamina ovate, $1.9-4.2 \times 1.5-2.6$ cm, obtuse at base, acute at apex, adaxially green to bluish green with greenish-white reticulations, grid lines thick, margin blurred, transverse ones 5–7, abaxially pale

green, 5–7-veined. Inflorescence a terminal raceme, laxly (6–)12–15-flowered, spirally arranged, pubescent; peduncle 4.0–6.5 cm long, pubescent, with 2–3 sheathing bracts; sheathing bracts 1.5– 1.9×0.6 -0.8 cm, oblong-lanceolate, acute at apex, pale green, clasping, more or less pubescent, especially on the margin, 3-5-veined; rachis 3.5-7 cm long, pubescent. Floral bracts ovatelanceolate, $1.4-1.7 \times 0.5-0.6$ cm, acuminate to acute at apex, pale green, longer than ovary, sometimes shortly ciliate at margins, pubescent abaxially, trichomes multicellular, up to 0.8 mm, glabrous adaxially, 3-veined. Flowers resupinate, opening weakly, 8-10 mm long. Sepals 1-veined, acute at apex, olive greenish, with reddish or brownish tinge when old, with dense clavate trichomes outside, trichomes up to 0.5 mm long; dorsal sepal ovate-lanceolate, $8-9 \times 2-3$ mm, forming hood with petals; lateral sepals ovate-lanceolate, $8-9 \times 3.5-4$ mm. Petals obliquely obovate-rhombic, 8.0- 8.5×3.0 –3.5 mm, acuminate to acute at apex, white, with reddish or brownish tinge at central part, glabrous, 1-veined. Labellum oblong-ovate, 6-7 mm long, yellow or yellowish; sac ca. 1.5-2.5 × 2.5–3.5 mm, with glandular hairs inside; blade oblong to oblong-lanceolate, entire, $5.0-6.5 \times 2.0-$ 3.0 mm, margins slightly undulate, obtuse to subacute at apex. Column ca. 1 mm long; rostellum 3.5–4.0 mm long, bifid, acuminate at apex. Stigma suborbicular, ca. 1×1 mm, entire. Anther cap yellowish brown, ovate, ca. 2.5×1.5 mm. Pollinarium 3–3.5 mm long; pollinia 2, oblong-obclavate, 0.8–1 mm long, dull yellowish white, sectile, bifid; caudicles 1.5–1.7 mm long; viscidium narrowly ovate-oblong, $2-3 \times ca$. 0.5 mm, acute at apex, membranous. Ovary terete, including pedicel 6.0– $7.5 \times ca. 2.0$ mm, pale green, twisted, pubescent.

Etymology. The species epithet is named after the type locality, Medog County.

Vernacular name. 墨脱斑叶兰 (Chinese pinyin: mò tuō bān yè lán).

Distribution and habitat. This species is currently known only from Medog County, Tibet, China. It grows under evergreen broad-leaved forests at elevations of 1600–2300 m.

Conservation status. During our three surveys in January, June and August 2021, *Goodyera medogensis* was found in the forests of Medog Town and Beibeng Township of Medog County with about more than 200 individuals. However, since the population assessment of this species in the whole Medog County has not been made, conservation status of this new species is best classified as 'Data Deficient' (DD) (IUCN Standards and Petitions Committee 2019).

Phenology. Flowering in July–August.

Additional specimens examined (paratypes). CHINA. Tibet Autonomous Region, Medog County, Bari village, elev. 1750 m, 23 June 2021, cultivated in East China Normal University, 10 August 2021 (fl.), *J. Huang & M. Sun 21062310* (HSNU); ibid., Medog village, 19 August 2021, *H. Z. Tian et al. 21081914* (HSNU); ibid., Gelin village, 21 August 2021, *H. Z. Tian et al. 21082102* (HSNU).

Discussion

According to Hu et al. (2016), sect. *Reticulum* is one of the four sections of *Goodyera*, and can be further divided into two subsections, viz., *G.* subsect. *Reticulum* S. W. Chung & C. H. Ou and *G.* subsect. *Foliosum* S. W. Chung & C. H. Ou. Most species of sect. *Reticulum* have reticulated venation on leaves and not opened lateral sepals. According to phylogenetic trees based on the three datasets in this study, although the sister group matter is still unclear for the conflicting results showed by nrDNA and cpDNA, position of the new species that belongs to subsect. *Reticulum* is confirmed. It is closer to *G. hemsleyana*, which is supported by the morphological and phylogenetic evidences.

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Figure 1. Phylogenetic tree of *Goodyera* species inferred by Bayesian analysis and maximum likelihood analyses based on the nrDNA (**a** ITS) and cpDNA (**b** matK + trnL-F) datasets. Posterior probability (PP) ≥ 0.50 in BI analysis and bootstrap (BS) values $\ge 50\%$ in ML analysis are indicated on the left and right of slanting bars above the branches respectively. Dashes means not supported, i.e. the BS value < 50% in the ML analysis or PP < 0.50 in the BI analysis. The samples of new species are highlighted in red, and other species of sect. *Reticulum* are highlighted in bold.



Figure 2. Phylogenetic tree of *Goodyera* species inferred by Bayesian analysis and maximum likelihood analyses based on the nr + cpDNA (including ITS, *matK* and *trnL-F*) datasets. Posterior probability (PP) ≥ 0.50 in BI analysis and bootstrap (BS) values $\ge 50\%$ in ML analysis are indicated on the left and right of slanting bars above the branches respectively. Dashes means not supported, i.e. the BS value < 50% in the ML analysis or PP < 0.50 in the BI analysis. The samples of new species are highlighted in red, and other species of sect. *Reticulum* are highlighted in bold.



Figure 3. *Goodyera medogensis*. A Habit B Inflorescence C Flower, lateral (left) and front (right) view, with the arrow showing close-up of trichomes on abaxial surface of sepals D Sepals E Petals
F Column with labellum, anther and ovary G Labellum H Pollinarium I Column with a part of ovary. Taken by Y. H. Tong.



Figure 4. *Goodyera medogensis.* **A** Habit **B** Leaf **C** Bract **D** Flower, front view **E** Flower, lateral view **F** Sepals **G** Petals **H** Column with labellum, anther and ovary **I** Labellum **J** Pollinarium **K** Column with a part of ovary. Scale bars: 3 cm (**A**); 2 cm (**B**); 5 mm (**C**–**I**); 4 mm (**K**); 2 mm (**J**).

Characters	G. medogensis	G. hemsleyana	
Grid lines of reticulations on adaxial surface of leaf blades	Thick, margin blurred, transverse ones fewer, 5–7	Thin, margin clear, transverse ones more, 7–17	
Number of flowers per inflorescence	(6–)12–15	4–10	
Floral bracts	Green	Pinkish green	
Trichomes on abaxial surface of floral bracts, sepals and ovary	Non-glandular	Glandular	
Sepal	Olive greenish	Pinkish green to pinkish white	
Petal	Obliquely obovate-rhombic; white, with reddish or brownish tinge at central part	Obliquely ovate-oblong; pinkish white	
Labellum	Yellow or yellowish, without lamella	White, with light greenish to pinkish tinge at apex; with a low bi-lamellate callus	

Table 1. Morphological comparison of Goodyera medogensis and G. hemsleyana.

Table S1 Details of materials for phylogenetic study.

	1 5 6	, <u> </u>				
Taxon	Voucher	Collector	Locality	nrITS	<i>trn</i> L-F	matK
Goodyera. araneosa	e023-1	Tian, Zhang & Zhou	Wangling, Sishuan, China	MN472742	MN544775	MN563606
Goodyera. araneosa	e023-2	Tian, Zhang & Zhou	Wangling, Sishuan, China	MN472743	MN544776	MN563607
Goodyera. biflora	359	Chung	Hsinchu, Taiwan, China	KT343986	KT385501	KT385588
Goodyera. biflora	a12	Tian	Xizang, China	KT343987	KT385498	KT385585
Goodyera. biflora	b03	Hu	Linan, Zhejiang, China	KT343988	KT385499	KT385586
Goodyera. biflora	b41	Hu&Li	Sangzhi, Hunan, China	KT343989	KT385500	KT385587
Goodyera bilamellata	183	Chung	Hualien, Taiwan, China	KT343990	KT385502	KT385589
Goodyera bilamellata	298	Chung	Taipei, Taiwan, China	KT343991	KT385503	KT385590
Goodyera bilamellata	628	Chung	Hsinchu, Taiwan, China	KT343992	KT385504	KT385591
Goodyera bilamellata	630	Chung	Nantou, Taiwan, China	KT343993	KT385505	KT385592
Goodyera bomiensis	588	Chung	Hsinchu, Taiwan, China	KT343994	KT385506	KT385593
Goodyera bomiensis	a15	Tian	Linzhi, Xizang, China	KT343995	KT385507	KT385594
Goodyera bomiensis	b129	Hu&Li	Huoshan, Anhui, China	KT343996	KT385508	KT385595
Goodyera bomiensis	c14	Tang	Xichang, Sichuan, China	KT343997	KT385509	KT385596
Goodyera	a11	Tian	Chayu, Xizang, China	KT343999	KT385510	KT385597
daibuzanensis						
Goodyera	b98	Tian	Pingdong, Taiwan, China	KT344000	KT385511	KT385598
daibuzanensis						
Goodyera foliosa	b12	Hu	Malipo, Yunnan, China	KT344004	KT385513	KT385600
Goodyera foliosa	b47	Hu	Guilin, Guangxi, China	KT344005	KT385514	KT385601
Goodyera foliosa	b80	Ye	Simao, Yunnan, China	KT344007	KT385516	KT385603
Goodyera fumata	443	Chung	Taipei, Taiwan, China	KT344010	KT385519	KT385606
Goodyera fumata	b125	Tian&Dong	Wuzhishan, Hainan,	KT344011	KT385520	KT385607

			China			
Goodyera fusca	e024	Jin	Yadong, Tibet, China	MK991804	MN013772	MN013778
Goodyera hachijoensis	46	Chung	Cibodas, Indonesia	-	KT385521	KT385608
Goodyera hemsleyana	b14	Hu	Malipo, Yunnan, China	KT344014	KT385522	KT385609
Goodyera henryi	a10	Tian	Cibagou, Xizang, China	KT344017	KT385523	KT385610
Goodyera henryi	b32	Hu	Wenchuan, Sichuan, China	KT344018	KT385524	KT385611
Goodyera henryi	b36	Hu & Li	Emeishan, Sichuan, China	KT344019	KT385525	KT385612
Goodyera hispida	b86	Tian	Motuo, Tibet, China	KT344020	KT385526	KT385613
Goodyera hispida	c12	Jiang		KT344021	KT3855227	KT385614
Goodyera	b16	Hu	Malipo, Yunnan, China	VT244025	VT285520	VT285616
kwangtungensis				K1344023	K1363329	K1303010
Goodyera	b30	Hu & Li	Nanchuan, Chongqing,	кт344027	KT385531	KT385618
kwangtungensis			China	K 1J 1 02/	K1565551	K 1505010
Goodyera	b44	Hu	Maoershan, Guangxi,	КТ344029	КТ385533	КТ385620
kwangtungensis			China	R 1511025	R1505555	R 1505020
Goodyera marginata	503	Chung	Chengdu, Sichuan, China	KT344030	KT385534	KT385621
Goodyera marginata	a13-4	Tian	Bomi, Tibet, China	KT344032	KT385535	KT385622
Goodyera marginata	b06	Hu	Dali, Yunnan, China	KT344033	KT385536	KT385623
Goodyera medogensis	21062209	Huang & Sun	Medog, Tibet, China	Waiting to		
				be assigned		
Goodyera medogensis	21062310	Huang & Sun	Medog, Tibet, China			
Goodyera medogensis	21062312	Huang & Sun	Medog, Tibet, China			
Goodyera medogensis	21062216	Huang & Sun	Medog, Tibet, China			
Goodyera nankoensis	b33	Hu & Li	Emeishan, Sichuan,	KT344036	KT385537	KT385624

Goodyera nankoensis	b57	Ge	Bomi, Xizang, China	KT344037	KT385538	KT385625
Goodyera pendula	525	Chung	Yilan, Taiwan, China	KT344039	KT385539	KT385626
Goodyera pendula	629	Chung	Taitong, Taiwan, China	KT344040	KT385540	KT385627
Goodyera pendula	a16	Tian	Nanling, Guangdong,	KT344041	KT385541	KT385628
			China			
Goodyera pendula	e038	Qin	Maoershan, Guangxi,	MK991805	MN013773	MN032023
			China			
Goodyera prainii	707_2	Tian & Hu	Yingjiang, Yunnan, China	KT344042	KT385542	KT385629
Goodyera procera	b01	Hu	Southern China Botanical	KT344044	KT385543	KT385630
			Garden, Guangdong			
Goodyera procera	b69	Tian & Dong	Wuzhishan, Hainan,	KT344045	KT385544	KT385631
			China			
Goodyera pusilla	a20	Tian	Ruyuan, Guangdong,	KT344046	KT385545	KT385632
			China			
Goodyera pusilla	b100	Tian	Taidong, Taiwan, China	KT344047	KT385546	KT385633
Goodyera repens	632	Chung	Deqing County, Tibet,	KT344048	KT385547	KT385634
			China			
Goodyera repens	a13-1	Tian	Bomi, Xizang, China	KT344049	KT385548	KT385635
Goodyera repens	b48	Wei	Mt.Tianshan, Xinjiang,	KT344050	KT385540	KT385636
			China	K1344030	K1383349	K1363030
Goodyera repens	b49	Wei	Mt.Tianshan, Xinjiang,	KT344051	KT385550	KT385637
			China			
Goodyera robusta	541	Chung	Mt. Fansipan, Sapa, Viet	KT344054	KT385552	KT385639
			Nam			
Goodyera rubicunda	b88	Tian	Bawangling, China	KT344060	KT385555	KT385642

China

Goodyera rubicunda	b101	Tian	Taidong, Taiwan, China	KT344058	KT385553	KT385640
Goodyera rubicunda	b104	Tian	Hualian, Taiwan, China	KT344059	KT385554	KT385641
Goodyera	a14	Tian	Linzhi, Xizang, China	VT244065	VT285556	VT285612
schlechtendaliana				K1344003	K1385550	K1303043
Goodyera	b85	Tian	Linzhi, Xizang, China	VT244067	VT295559	VT285645
schlechtendaliana				K1344007	K1303550	K1303043
Goodyera	b106	Tian	Taoyuan, Taiwan, China	VT244066	VT285557	VT285611
schlechtendaliana				K1344000	K1303337	K1303044
Goodyera	109	Chung	Yilan, Taiwan, China	VT244069	VT295560	VT295617
seikomontana				K1344000	K1383300	K130304/
Goodyera	a22	Tian	Nanling, Guangdong,	VT244060	VT285550	VT285616
seikomontana			China	K1344009	K1303333	K1303040
Goodyera thailandica	b77	Ye	Simao, Yunnan, China	KT344070	KT385561	KT385648
Goodyera velutina	b04	Hu	Tianmu mountain,	KT344073	KT385567	KT385640
			Zhejiang, China	K1344073	K1383302	K13030 4 9
Goodyera velutina	b31	Hu&Li	Sanjiang Ecological			
			Tourist Park, Sichuan,	KT344075	KT385564	KT385651
			China			
Goodyera velutina	b105	Tian	Taoyuan County, Taiwan,	VT244074	VT295562	VT295650
			China	K13440/4	K1383303	K1303030
Goodyera viridiflora	524	Chung	XinZhu, Taiwan, China	KT344080	KT385567	KT385654
Goodyera viridiflora	a19	Tian	Nanling, Guangdong,	VT244092	VT205560	VT295655
			China	K1344062	K1303300	K1303033
Goodyera viridiflora	b19	Hu	Malipo, Yunnan, China	KT344084	KT385570	KT385657
Goodyera viridiflora	b107	Chung	Philippines	KT344083	KT385569	KT385656
Goodyera vittata	706	Tian & Hu	Yingjing, Yunnan, China	KT344086	KT385572	KT385659

Goodyera wolongensis	c13	Tang	Wanglang, Sichuan,	KT344087	KT385573	KT385660
			China			
Goodyera wolongensis	e020-1	Tian, Zhang & Zhou	Songpan, Sichuan, China	MK991806	MN013779	MN013774
Goodyera wolongensis	e021-4	Tian, Zhang & Zhou	Pingwu, Sichuan, China	MK991807	MN013780	MN013775
Goodyera yamiana	78	Chung	Taidong, Taiwan, China	KT344088	KT385574	KT385661
Goodyera yunnanensis	a13-2	Tian	Bomi, Tibet, China	KT344089	KT385575	KT385662
Goodyera yunnanensis	a13-3	Tian	Bomi, Tibet, China	KT344090	KT385576	KT385663
Pachyplectron arifolium	TJM1171	Motley	_	E1472224	E1571206	
	NY			1'J4/3334	1'J3/1280	_

Note: "_" means missing data.

Table. S2 Primers for sequence amplication and sequencing

Sequenc	Primers (5'-3')	Reference	Amplication protocol
e			
ITS	18S dir: CGTAACAAGGTTTCCGTAGG	Venora et al., 2000 [1]	92°C 3min; 95°C 30s, 50°C 30s, 72°C
	ITS4: TCCTCCGCTTATTGATATGC	White et al., 1990 ^[2]	1min, 30cycles; 72°C 7min
<i>trn</i> L-F	c: CGAAATCGGTAGACGCTACG	Taberlet et al., 1991 [3]	94°C 2min; 94°C 1min, 50°C 30s,
	f: ATTTGAACTGGTGACACGAG	Taberlet et al., 1991 ^[3]	72°C 1min, 30cycles; 72°C 7min
matK 1	19F: CGTTCTGACCATATTGCACTATG	Molvray et al., 2000 [4]	80°C 5min; 95°C 1min, 51°C 1min,
	834R: AAAGACTCCARAAGATRTTG	Kocyan et al., 2004 ^[5]	65°C 4min, 30cycles; 65°C 5min
matK 2	731F: TCTGGAGTCTTTCTTGAGCGA	Gervendeel et al., 2001 ^[6]	The same as <i>mat</i> K 1
	trnK2R: AACTAGTCGGATGGAGTAG)	Johnson & Soltis, 1994 ^[7]	

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