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ORIGINAL ARTICLE

Secondary contact, hybridization and polyploidization add to the biodiversity in the Hengduan Mountains, exemplified by the widespread *Corallodiscus lanuginosus* (Gesneriaceae)

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Abstract *Corallodiscus lanuginosus* is distributed 12,000 km across its northeast–southwest boundaries, showing its highest morphological diversity in the Hengduan Mountains, Southwest China. We investigated the mechanisms behind the high diversity in this species. We acquired ITS sequence data across the distribution range of the genus, reconstructed dated phylogenies, projected the resulting clades onto geographic maps and linked the results with inferred ploidy levels of the populations. We found strong geographic patterns for the ITS clades but with extensively overlapping ranges, particularly in the Hengduan Mountains area. We deduced an origin of *C. lanuginosus* in the mid-Oligocene. We used the presence of ITS polymorphisms to infer a high level of hybridization events mainly in areas of clade overlap, where we also inferred repeated tetraploidization events to have taken place. Pre- and post-Quaternary climate oscillation-driven expansions and contractions of distribution ranges have allowed secondary contacts, hybridization and introgression that shaped the current patchy distribution of morphological diversity in the species. Polyploidization as a reinforcing genetic barrier added to the genetic diversity in space and time particularly in the Hengduan Mountains.

Keywords *Corallodiscus* · Gesneriaceae · Hengduan Mountains · Hybridization · Polyploidization · Secondary contacts

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Introduction

Orogenic events such as the rise of the Himalayas and the uplift of the Qinghai-Tibetan Plateau (QTP) and their associated climatic effects can provide new ecological niches and opportunities for the accumulation of diversity through allopatric speciation (e.g. Ruddiman and Kutzbach 1989; Shi et al. 2001) and ecological speciation (e.g. Liu et al. 2013; Gao et al. 2015; Wang et al. 2015). Climatic fluctuations during the Quaternary (e.g. Wang et al. 2010; Xu et al. 2010) can add to the diversity by expanding and contracting distribution ranges, local adaptation, allowing secondary contacts and creating, over time, biodiversity hot spots such as the Himalayas and Hengduan Mountains (Myers et al. 2000; Liu and Tian 2007; Li et al. 2011).

Secondary contacts may lead to hybridization, introgression and hybrid swarms with plants of mixed or continuous morphological characters (e.g. Stebbins 1959; Rieseberg and Wendel 1993; Gao et al. 2015). The levels of backcrossing and introgression can influence the genetic diversity of resulting lineages including the transfer of adaptive ecological traits resulting in local ecotypes (Lewontin and Birch

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1966; Bleeker 2003; Seehausen 2004), allowing the expansion into new areas and niches (Cruzan and Arnold 1993; Neuffer et al. 1999; Ellstrand and Schierenbeck 2000) and the reinforcement or breakdown of isolating mechanisms. The latter can cause difficulties in taxonomic delineations (e.g. Wendel and Grover 2015). The former often involves polyploidization, even repeatedly so, as a consequence of hybridization between diploid lineages (e.g. Soltis et al. 2004; and references therein; Paterson et al. 2012). Polyploidy plays a major role in plant evolution with an estimated one-third to 70% of plant speciation involving polyploidization somewhere along their evolutionary trajectories (Stebbins 1971; Levin 2002; Rieseberg and Willis 2007; Weiss-Schneeweiss et al. 2013).

The genus *Corallodiscus* Batalin resides on an early diverging branch of tribe Trichosporeae in the predominantly Old World subfamily Didymocarpoideae of Gesneriaceae (Möller et al. 2009; Weber et al. 2013; Roalson and Roberts 2016). The genus has received various systematic treatments since it was established. Currently, *Corallodiscus* includes three species in China, *C. conchifolius* Batalin, *C. kingianus* (Craib) B.L.Burtt and *C. lanuginosus* (Wall. ex DC.) B.L.Burtt (Wang et al. 1998). *Corallodiscus lanuginosus* has an extremely wide distribution range, from Beijing Municipality in Northeast China to the Western Ghats in India, some 12,000 km in northeast–southwest direction, and reaches northern Thailand in the South (Wang et al. 1998; Kamble et al. 2006; Rout et al. 2008). Not surprisingly, there exists great morphological diversity within the species particularly around the eastern Himalayas and Hengduan Mountains, an area of extreme species richness (e.g. Liu et al. 2013). This diversity was previously ascribed to several species which exhibited continuous and overlapping variation in leaf and flower characters without geographic patterns, and were thus amalgamated into one variable species, *C. lanuginosus* (Burtt 1947; Wang et al. 1990, 1998).

In the present work, we studied the evolutionary mechanisms responsible for the high morphological diversity found in *C. lanuginosus*, particularly in the Hengduan Mountains. Several studies focussed on mechanisms adding to the biodiversity richness here, such as ecological niche adaptation and allopatric speciation (Shi et al. 2001; Liu et al. 2013; Gao et al. 2015; Wang et al. 2015). Here, we focus on the importance of hybridization and polyploidy in this scenario. The two chromosome counts available for *C. lanuginosus* of $2n = 20$ and 40 (Möller and Pullan 2015 onwards) indicate that variation at the ploidy level exists in the species.

To this end, we investigated the genetic landscape of *C. lanuginosus* by sampling 116 locations across the distribution of *Corallodiscus* in China and adjacent regions and obtained molecular data of the nuclear-transcribed spacer region (ITS) to infer phylogenetic relationships among the samples and projected this diversity onto a geographic map. This will illustrate the link between the relatedness of the populations and their geographic distribution. A dated phylogeny will provide the time frame for the scenario. We further use data from cytology and pollen sizes to infer the occurrence and distribution of ploidy across the localities. Our results add to our understanding of the interplay of mechanisms of diversity accumulation in the biodiversity hot spot of the Hengduan Mountains in Southwest China (Fig. 1).

Materials and methods

Plant materials

Material for experimental work was collected in the field or from herbarium specimens between 2009 and 2012 representing 116 localities from across the distribution range of *Corallodiscus* in China and adjacent regions in India, Nepal and Thailand, at locations determined from specimen sheets held at herbaria of E, KUN, PE, XTBG (Online Resource 1, Fig. 1). Ten locations for *C. kingianus* and 14 of *C. conchifolius* were included for comparison to *C. lanuginosus*. For 26 locations of *C. lanuginosus* and *C. kingianus*, multiple individuals (2–5) were included to test the homogeneity of the ITS sequences (i.e. number of ITS types) within populations (Table 1). In total, samples from 182 plants were included. Three samples were included as outgroup for the phylogenetic analysis, based on previous work (Möller et al. 2009). These were *Leptoboea multiflora*, *Tetraphyllum roseum* and *Jerdonia indica*.

DNA extraction and PCR amplification

For DNA extraction, leaves dried in silica gel (from field collections) and from herbarium specimens were used. Genomic DNA was extracted through a modified CTAB procedure (Doyle and Doyle 1987) with no further purification.

The complete ITS region was PCR amplified using primers "ITS-5P" (5′-GGA AGG AGA AGT CGT AAC AAG G-3′) (Möller and Cronk 1997) and "ITS-4" (5′-TCC TCC GCT TAT TGA TAT GC-3′) (White et al. 1990) or "ITS-5P" and "ITS-8P" (5′-CAC GCT TCT CCA GAC TAC A-3′) (Möller and Cronk 1997). For some samples with length polymorphisms, primers residing in the 5.8S gene were applied, which were "ITS-2G" (5′-GTG ACG CCC AGG CAG ACG T-3′) and "ITS-3P" (5′-GCA TCG ATG AAG AAC GTA GC-3′) which were used in combination with "ITS-5P" and "ITS-8P", respectively (Möller and Cronk 1997). PCR was performed in volumes of 25 μL with the following final concentrations: 2.5 μL 10× buffer, 1.5 μL Mg^{2+} (25 mmol/L), 1.5 μL of dNTP (2.5 mmol/L), 1 μL of each primer (10 mmol/L), 0.2 μL

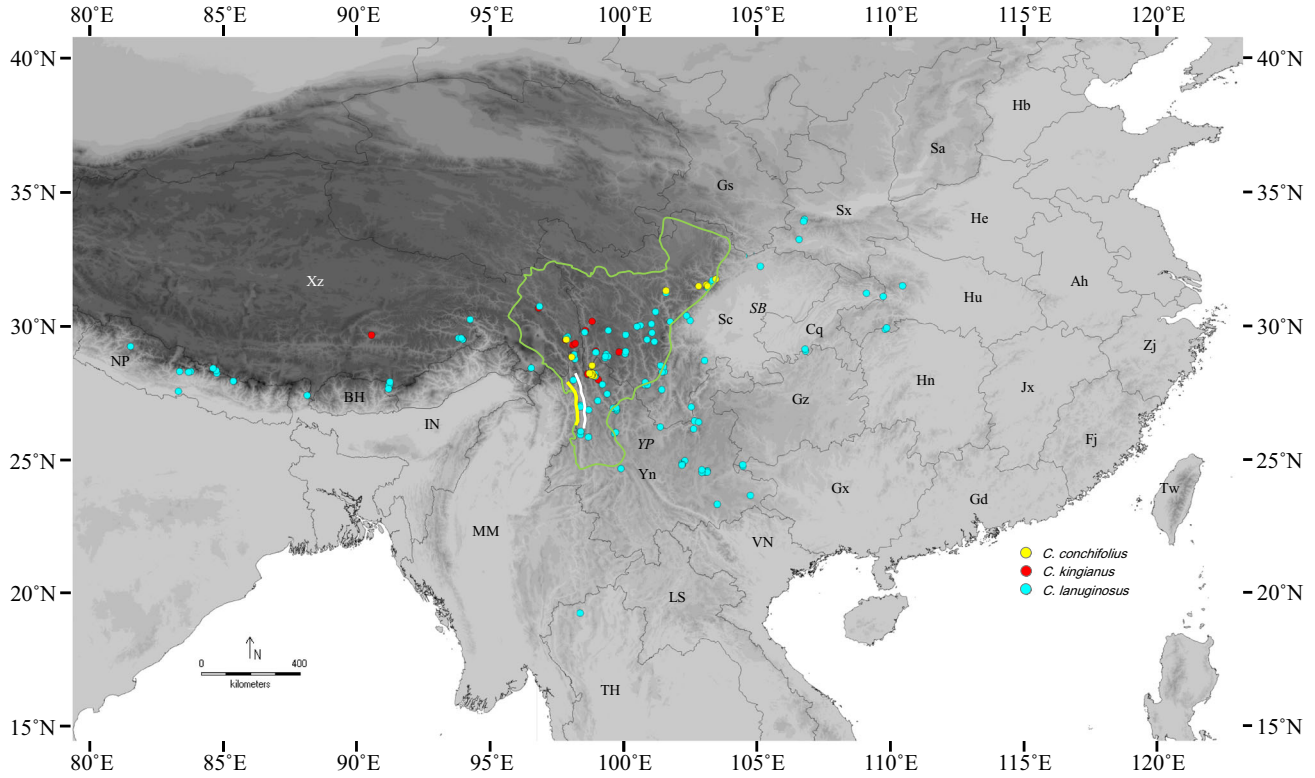


Fig. 1 Localities of samples included in the present study with names according to Wang et al. (1998). Hengduan Mountains region *outlined in green*; *yellow line*: Gaoligong Shan; *white line*: Nu Shan. Country and province abbreviations: Ah, Anhui; Cq, Chongqing; Fj, Fujian; Gd, Guangdong; Gs, Gansu; Gx, Guangxi; Gz, Guizhou; Hb, Hebei, He, Henan; Hn, Hunan; Hu, Hubei; Jx, Jiangxi; Sa, Shanxi; Sc, Sichuan; Sx, Shaanxi: Xz, Xizang; Yn, Yunnan; Zj, Zhejiang; Tw, Taiwan. BH, Bhutan; IN, India; LS, Laos; MM, Myanmar; NP, Nepal; TL, Thailand; VN, Vietnam. *SB*, Sichuan basin; *YP*, Yunnan plateau

Taq-Polymerase (TaKaRa, Japan), 15.3 µL ddH_2O and 2 µL template DNA. The PCR profile consisted of 3-min initial denaturation at 94 °C, thereafter 30 cycles, each consisting of 1-min denaturation at 94 °C, 1-min annealing at 55 °C and 1.5-min extension at 72 °C, and finished with a final extension of 5 min at 72 °C. The PCR products were checked by electrophoresis on 1% agarose gels and Sanger sequenced (BGI, Shenzhen, China).

The sequencing primers were the same as used for PCR. For several samples, additionally internal primers "ITS-2G" and "ITS-3P" were used. The sequences were checked and assembled in Sequencher v.5.3 (Gene Codes Corporation, Ann Arbor, MI, USA) and aligned in SeqMan v.7.1.0 (44.1) (Lasergene, DNASTAR Inc.). All newly acquired sequences have been submitted to GenBank (Online Resource 1) and the alignment matrix to TreeBASE (study accession number: TB2:S20267).

Phylogenetic analyses

A maximum parsimony analysis was carried out using PAUP v.4.10b (Swofford 2002) on unordered and unweighted characters with polymorphic sites treated as base polymorphisms. In the light of the large number of taxa, PAUPRatchet (Nixon 1999) with default settings was used to obtain starting trees, which were further optimized by TBR, with MulTrees and Steepest Descent on. Clade support was obtained as 10,000 bootstrap replicates of random additions with TBR on and MulTrees off (Möller et al. 2009).

A Bayesian inference analysis was carried out using MrBayes v.3.2.4 (Ronquist et al. 2012). Models were selected in MrModeltest (Nylander 2004) independently for the ITS spacers and the 5.8S gene and were GTR + G and K80 + I, respectively. Two independent runs of four MCMC chains were run over five million generations and a tree sampled every 1000th generation (=5000 trees). The first 250 trees were discarded as burn-in, and from the remaining trees a majority rule consensus Bayesian inference tree obtained including clade posterior probabilities (PP). Diagnostics for the runs were obtained in Are We There Yet (Wilgenbusch et al. 2004) (Online Resource 2).

Divergence time estimations

To estimate the ages of *Corallodiscus* lineages, we used BEAST v.1.8.3 (Drummond and Rambaut 2007) on the

Table 1 Populations of *Corallodiscus lanuginosus* and *C. kingianus* with multiple samples included in the study showing one or several ITS types

DNA ID	Population code	*N*	ITS types	Member of clade (no. of plants) [putative cross with population clade]
I-18	YJL	2	1	II-1a [clean]
I-56	RPB	2	1	II-1a [clean]
I-76	05-534	2	1	II-2b [clean]
I-4	LN	5	2	II-2c (3) [clean],
				II-2c (2) [clean]
I-6	XCY	5	1	II-2c [clean]
I-10	LGX	5	1	II-2c [clean]
I-105	NBZL	4	1	II-2c [clean]
I-130	DQS	4	1	II-2c [clean]
I-54	LZF	5	4	II-1a (1) [×II-3b],
				II-3a (1) [×?],
				II-3a (1) [×?],
				II-3b (2) [×II-3b]
I-71	BBL	2	1	II-3a [×?]
I-3	YTK	2	1	II-3a (2) [×?]
I-27	NPH	2	1	II-3b [clean]
I-48	XCL	4	4	II-3b (1) [×II-3b],
				II-3b (1) [×II-3b],
				II-3b (1) [clean],
				III-1b (1) [×II-3b]
I-106	KM	2	2	II-2c (1) [clean],
				III-4a (1) [×III-4]
I-23	XBB	2	1	III-1a [×III-?]
I-58	MKK	3	2	III-1b (1) [×II?],
				III-1b (2) [×?]
I-66	NiXi	5	1	III-1b [clean]
I-73	km1892	5	1	III-1b [clean]
I-33	Dali64	2	2	III-2 (1) [×III-4?],
				III-2 (1) [×III-4?]
I-100	YZ	5	2	III-2 (4) [×III-2],
				III-2 (1) [×III-2]
I-2	DLL	5	1	III-3 [clean]
I-86	MB	5	1	III-4a [clean]
I-41	09-1458	5	3	III-2 (1) [clean],
				III-4a (1) [clean],
				III-4a (3) [×III-2]
I-26	XGH	2	2	III-4a (1) [×III-4b],
				III-4b (1) [×III-4b]
I-40	MTD	2	2	III-4b (1) [×III-4?],
				III-4b (1) [×III-4?]
I-43	LJA	5	2	III-4b (4) [×III-4a],
				III-4b (1) [×III-4b]
Sum		92	42	

N—number of plants analysed per population. Column at end lists clade affiliation of the ITS types with number of individuals in round brackets and indicated partner clade in inferred crosses in square brackets; clean—indicates absence of polymorphisms in the electropherograms of individuals

ITS data matrix. There are no fossils available for *Corallodiscus* or Gesneriaceae, but a dated phylogeny of Gesneriaceae including *Corallodiscus* samples (Petrova et al. 2015). Thus, we used secondary calibration points, (a) the split between *Jerdonia* and the remaining Trichosporeae of 55.9 MY (95% HPD: 45.47–67.0), (b) the split between *Corallodiscus* and *Tetraphyllum* and *Leptoboea* of 47.59 MY (95% HPD: 36.97–58.15) and (c) the split between *C. conchifolius* and *C. lanuginosus* of 22.15 MY (95% HPD: 7.08–37.95). A Yule prior for the tree topology and a normal prior was applied to the calibration points (for a and b: SD = 5, for c: SD = 6). The analysis was run for 10 million generations with a lognormal relaxed molecular clock and models of DNA evolution as described above. Twelve MCMC chains were run independently and sampled every 2000th generation in BEAST. Convergence of the runs was tested in TRACER v.1.5 (Drummond and Rambaut 2007), with the effective sample size (ESS) > 200 for each parameter. A metric tree was generated in FigTree v.1.4 (Rambaut 2012).

Inference of ploidy level

Corallodiscus lanuginosus plants are difficult to cultivate for chromosome studies. To obtain an indication of the ploidy level across the range of populations, the chromosome number of 14 plants from five populations could be determined (Online Resource 3) and related to the pollen diameter of those five populations and an additional 41 populations (Online Resource 4) and the populations assigned a ploidy-level status accordingly.

Chromosome counts

Chromosome counts for *C. lanuginosus* followed the method described in Jong and Möller (2000). In brief, after harvesting, the root tips were pre-treated with 8-hydroxyquinoline at room temperature for 4–5 h in the dark, fixed in Farmer's solution, hydrolysed in 5 N HCl for 30 min, stained with Feulgen reagent for 2 h in the dark, softened in a mixture of 4% cellulose and 4% pectinase for 20–30 min at 36 °C and squash preparations made.

Pollen diameter measurements

Anthers of flowers fixed in FAA (5 mL formaldehyde, 90 mL 70% ethanol, 5 mL acetic acid) were dissected and pollen extracted on a microscope slide in 1% acetocarmine. Images were taken on a compound microscope (Leica DM1000) (Leica, Wetzlar, Germany), the diameter of 5–10 pollen grains per sample measured, and mean and SD values determined. Flowers of 46 populations across the sample range of *C. lanuginosus* were analysed (Online Resources 3, 4). Since ploidy level is often closely correlated with pollen size (Knight et al. 2010) and flower organ size (Bennett 1971; Balao et al. 2011), we plotted the pollen size against the corolla length.

Results

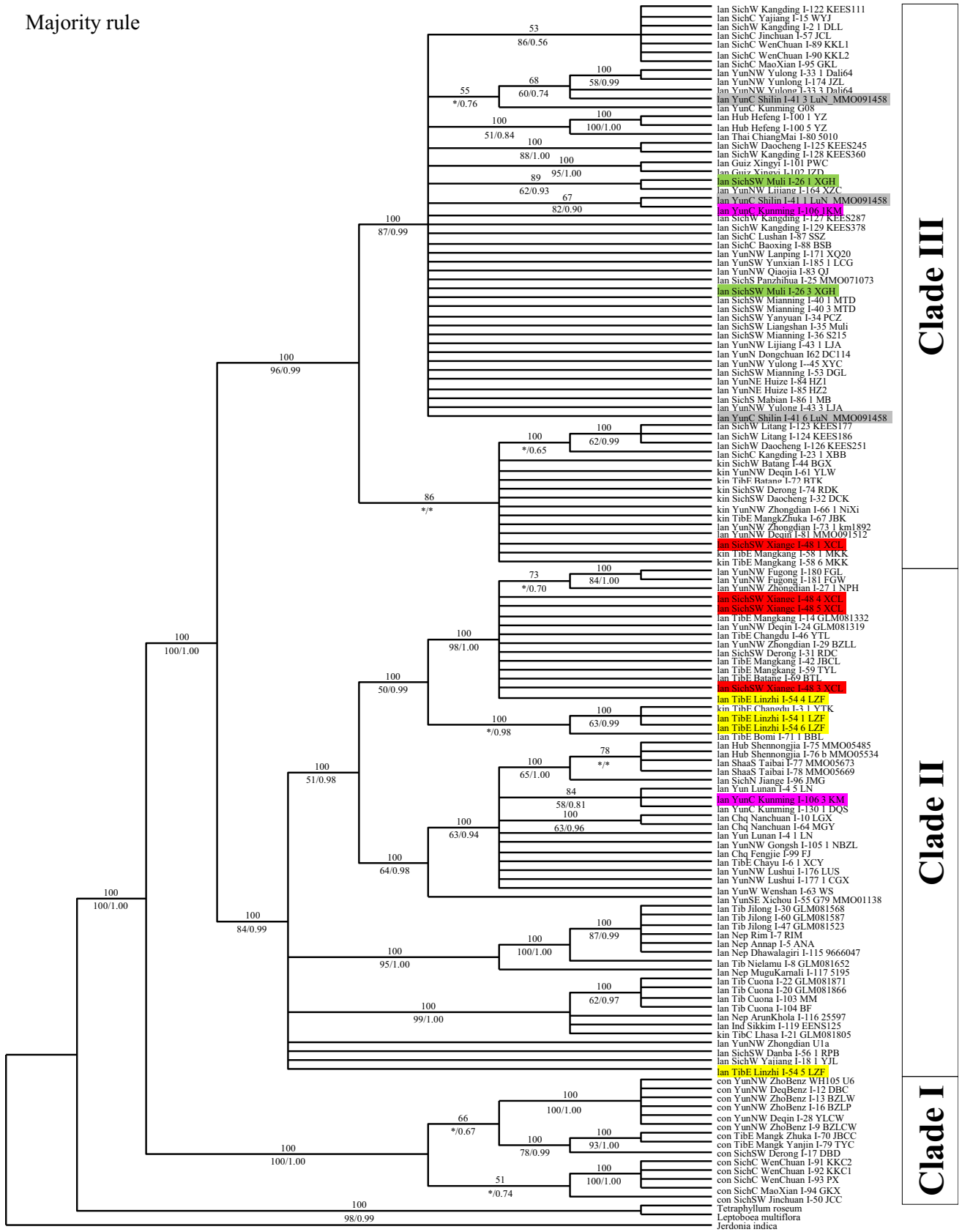
ITS population homogeneity

Among the 26 populations with multiple samples included, 15 (58%) were monomorphic in their ITS sequences, with the remaining 11 populations possessing 2–4 different ITS types (Table 1).

Phylogenetic analyses

For populations with multiple samples tested, all unique ITS sequence types were included in the phylogenetic analysis, and for those samples that were identical within a population only one sequence per population was used in the analysis. The aligned ITS matrix thus included 132 *Corallodiscus* ITS sequences, of which 11 came from *C. kingianus* and 107 from *C. lanuginosus*, 14 from *C. conchifolius*, plus the three outgroup samples. The matrix was 703 characters long and included 356 (50.6%) constant characters, 110 (15.6%) parsimony uninformative and 237 (33.8%) parsimony informative characters. The parsimony analysis retrieved 82,219 trees of 612 steps length, with a high consistency index (CI) of 0.8121 and high retention index (RI) of 0.9631. The majority rule consensus tree was fully congruent with the Bayesian tree. The genus *Corallodiscus* was found highly supported (BS = 100%; PP = 1.00). Within it, three strongly supported main clades were retrieved, one (Clade I) comprising the *C. conchifolius* samples (BS = 100%; PP = 1.00) which was sister to the other two main clades (BS = 100%; PP = 1.00). Each of these sister clades was well supported, Clade II with BS = 84% and PP = 0.99 and Clade III with BS = 96% and PP = 0.99. The *C. kingianus* samples scattered among the *C. lanuginosus* samples predominantly in Clade III with only one in Clade II. Within these two main clades several levels of subclades were retrieved with varying strength of branch support generally decreasing from the backbone towards the tips (Fig. 2).

Of those populations with multiple ITS types, most had types occurring within the same subclades, except for population "LuN", "LZF" and "XGH", where the different types fell in different subclades within Clade II ("LZF") or Clade III ("LuN", "XGH"). Populations "KM" and "XCL" possessed types falling in separate main clades II and III (Fig. 2; Table 1).


Majority rule
lan SichW Kangding I-122 KEES111
lan SichC Yajiang I-15 WYJ
lan SichW Kangding I-2 1 DLL
lan SichC Jinchuan I-57 JCL
lan SichC WenChuan I-89 KKL1
lan SichC WenChuan I-90 KKL2
lan SichC MaoXian I-95 GKL
lan YunNW Yulong I-33 1 Dali64
lan YunNW Yunlong I-174 JZL
lan YunNW Yulong I-33 3 Dali64
lan YunC Shilin I-41 3 LuN_MMO091458
lan YunC Kunming G08
lan Hub Hefeng I-100 1 YZ
lan Hub Hefeng I-100 5 YZ
lan Thai ChiangMai I-80 5010
lan SichW Daocheng I-125 KEES245
lan SichW Kangding I-128 KEES360
lan Guiz Xingyi I-101 PWC
lan Guiz Xingyi I-102 JZD
lan SichSW Muli I-26 1 XGH
lan YunNW Lijiang I-164 XZC
lan YunC Shilin I-41 1 LuN_MMO091458
lan YunC Kunming I-106 1 KM
lan SichW Kangding I-127 KEES287
lan SichW Kangding I-129 KEES378
lan SichC Lushan I-87 SSZ
lan SichC Baoxing I-88 BSB
lan YunNW Lanping I-171 XQ20
lan YunSW Yunxian I-185 1 LCG
lan YunNW Qiaojia I-83 QJ
lan SichS Panzhihua I-25 MMO071073
lan SichSW Muli I-26 3 XGH
lan SichSW Mianning I-40 1 MTD
lan SichSW Mianning I-40 3 MTD
lan SichSW Yanyuan I-34 PCZ
lan SichSW Liangshan I-35 Muli
lan SichSW Mianning I-36 S215
lan YunNW Lijiang I-43 1 LJA
lan YunN Dongchuan I62 DC114
lan YunNW Yulong I--45 XYC
lan SichSW Mianning I-53 DGL
lan YunNE Huize I-84 HZ1
lan YunNE Huize I-85 HZ2
lan SichS Mabian I-86 1 MB
lan YunNW Yulong I-43 3 LJA
lan YunC Shilin I-41 6 LuN_MMO091458
lan SichW Litang I-123 KEES177
lan SichW Litang I-124 KEES186
lan SichW Daocheng I-126 KEES251
lan SichC Kangding I-23 1 XBB
kin SichW Batang I-44 BGX
kin YunNW Deqin I-61 YLW
kin TibE Batang I-77 BTK
kin SichSW Derong I-74 RDK
kin SichSW Daocheng I-32 DCK
kin YunNW Zhongdian I-66 1 NiXi
kin TibE MangkZhuka I-67 JBK
lan YunNW Zhongdian I-73 1 km1892
lan YunNW Deqin I-81 MMO091512
lan SichSW Xiange I-48 1 XCL
kin TibE Mangkang I-58 1 MKK
kin TibE Mangkang I-58 6 MKK
lan YunNW Fugong I-180 FGL
lan YunNW Fugong I-181 FGW
lan YunNW Zhongdian I-27 1 NPH
lan SichSW Xiange I-48 4 XCL
lan SichSW Xiange I-48 5 XCL
lan TibE Mangkang I-14 GLM081332
lan YunNW Deqin I-24 GLM081319
lan TibE Changdu I-46 YTL
lan YunNW Zhongdian I-29 BZLL
lan SichSW Derong I-31 RDC
lan TibE Mangkang I-42 JBCL
lan TibE Mangkang I-59 TYL
lan TibE Batang I-69 BTL
lan SichSW Xiange I-48 3 XCL
lan TibE Linzhi I-54 4 LZF
kin TibE Changdu I-3 1 YTK
lan TibE Linzhi I-54 1 LZF
lan TibE Linzhi I-54 6 LZF
lan TibE Bomi I-71 1 BBL
lan Hub Shennongjia I-75 MMO05485
lan Hub Shennongjia I-76 b MMO05534
lan ShaaS Taibai I-77 MMO05673
lan ShaaS Taibai I-78 MMO05669
lan SichN Jiange I-96 JMG
lan Yun Lunan I-4 5 LN
lan YunC Kunming I-106 3 KM
lan YunC Kunming I-130 1 DQS
lan Chq Nanchuan I-10 LGX
lan Chq Nanchuan I-64 MGY
lan Yun Lunan I-4 1 LN
lan YunNW Gongsh I-105 1 NBZL
lan Chq Fengjie I-99 FJ
lan TibE Chayu I-6 1 XCY
lan YunNW Lushui I-176 LUS
lan YunNW Lushui I-177 1 CGX
lan YunW Wenshan I-63 WS
lan YunSE Xichou I-55 G79 MMO01138
lan Tib Jilong I-30 GLM081568
lan Tib Jilong I-60 GLM081587
lan Tib Jilong I-47 GLM081523
lan Nep Rim I-7 RIM
lan Nep Annap I-5 ANA
lan Nep Dhawalagiri I-115 9666047
lan Tib Nielamu I-8 GLM081652
lan Nep MuguKarnali I-117 5195
lan Tib Cuona I-22 GLM081871
lan Tib Cuona I-20 GLM081866
lan Tib Cuona I-103 MM
lan Tib Cuona I-104 BF
lan Nep ArunKhola I-116 25597
lan Ind Sikkim I-119 EENS125
kin TibC Lhasa I-21 GLM081805
lan YunNW Zhongdian U1a
lan SichSW Danba I-56 1 RPB
lan SichW Yajiang I-18 1 YJL
lan TibE Linzhi I-54 5 LZF
con YunNW ZhoBenz WH105 U6
con YunNW DeqBenz I-12 DBC
con YunNW ZhoBenz I-13 BZLW
con YunNW ZhoBenz I-16 BZLP
con YunNW Deqin I-28 YLCW
con YunNW ZhoBenz I-9 BZLCW
con TibE Mangk Zhuka I-70 JBCC
con TibE Mangk Yanjin I-79 TYC
con SichSW Derong I-17 DBD
con SichC WenChuan I-91 KKC2
con SichC WenChuan I-92 KKC1
con SichC WenChuan I-93 PX
con SichC MaoXian I-94 GKX
con SichSW Jinchuan I-50 JCC
Tetraphyllum roseum
Leptoboea multiflora
Jerdonia indica
Clade III
Clade II
Clade I

◀**Fig. 2** Maximum parsimony majority rule consensus tree of 82,219 most parsimonious trees of 612 steps length for 132 *Corallodiscus* samples based on internal transcribed spacer sequences of ribosomal DNA (consistency index: 0.8121; retention index: 0.9631; rescaled consistency index: 0.7821). Identical *colours indicate* samples from the same population. con = *C. conchifolius*, kin = *C. kingianus*, lan = *C. lanuginosus*

The clades identified within *C. lanuginosus* reveal a strong geographic component (Fig. 3), with Clade III-1, a high-altitude clade (Table 2), residing entirely in the Hengduan Mountains, Clade III-3 and III-4 occupying midrange altitudes in the Hengduan Mountains along the western edge of the Sichuan basin and on the Yunnan Plateau, respectively, while Clade III-2 occupies lower-altitude areas on the southern edges of the Hengduan Mountains and on the Yunnan Plateau, where it overlaps with Clade III-4, to the leading edge of the species in the East reaching Hunan and the South reaching as far as Thailand. Two of the three subclades of Clade II have the widest distribution ranges, particularly Clade II-2 that reaches from the Hengduan Mountains around the Sichuan basin as far as Hubei of the Central China region. Whether this clade reaches the furthest eastern populations of *Corallodiscus* in Hebei is feasible but requires further sampling there. Clade II-1 spans from the western fringes of the Hengduan Mountains westwards into the Himalayas and eastwards to the western fringes of the Sichuan basin where it overlaps with Clade III-3, while Clade II-3 is mainly of central Hengduan Mountain distribution but spreads westwards reaching Linzhi ("LZF") in the East Himalayas with considerable overlap with clades II-1 and III-1.

Polymorphic ITS sites

Of the 118 samples of *C. lanuginosus* (henceforth including *C. kingianus*), 64 showed no ITS base polymorphisms,

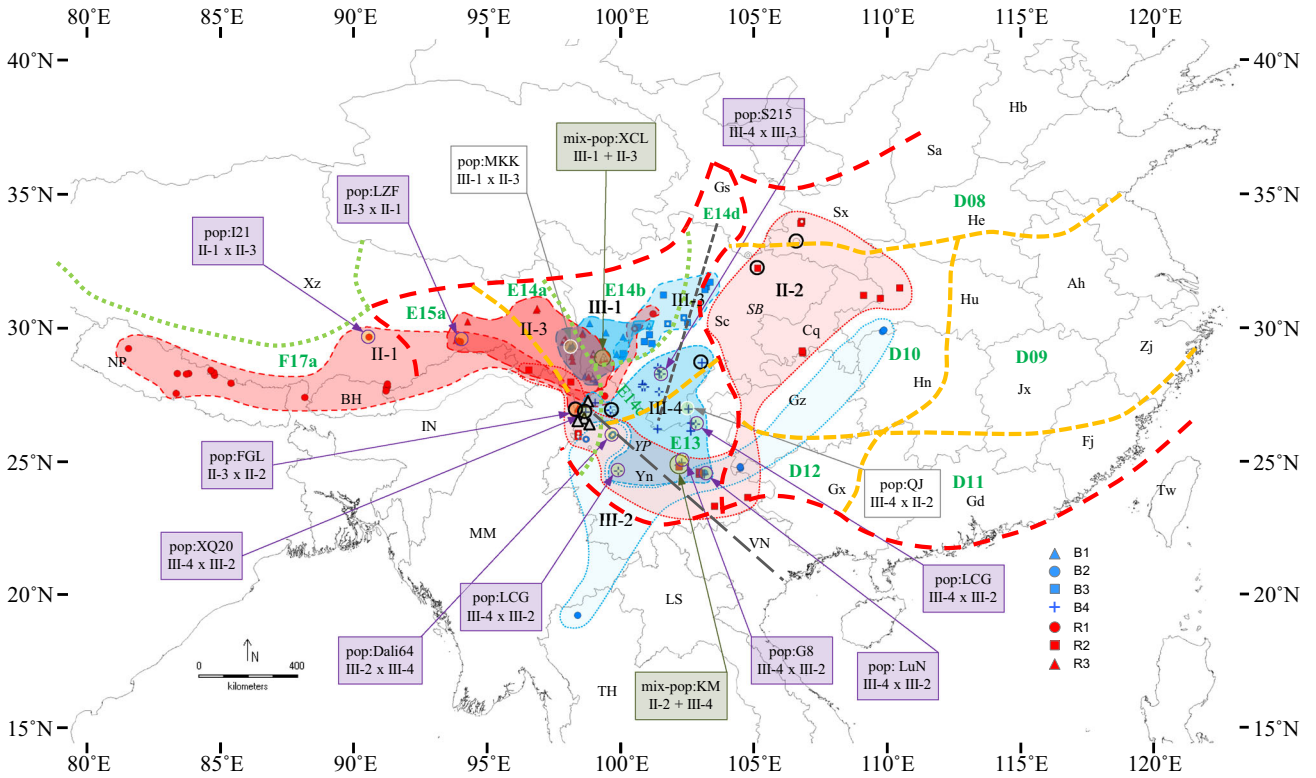


Fig. 3 Distribution of ITS clades of samples of *Corallodiscus lanuginosus*. Clade II (*shaded red*), Clade III (*shaded blue*). Populations *circled black*—tetraploids with ITS data, and populations with *black triangle* tetraploids with ITS too polymorphic to sequence. Populations with inferred hybrids within main clades (*purple-shaded boxes*), with inferred hybrids between main clades (*unshaded box*), with mixed ITS types between Clade II and III (*green-shaded boxes*), *dark grey-dashed lines* = Tanaka-Kaiyong line after Li and Li (1992, 1997). *Red* (*dashed lines*), *orange* (*dotted lines*) and *green* (*square boxes*) *lines* are phytogeographic regions after (Wu and Wu, 1998). Codes: D, Sino-Japanese Forest subkingdom; D8, North China region; D9, East China region; D10, Central China; D11, South China mountain region; D12, Yunnan. Guizhou and Guangxi limestone mountain and hill region: E, Sino-Himalayan forest subkingdom; E13, Yunnan Plateau region; E14, Hengduan Mountains region; E14a, Three River gorges subregion; E14b, North Hengduan Mountains subregion; E14c, South Hengduan Mountains subregion; E14d, Taohe-Mingshan Mountain subregion; E15a, Southeast Xizang subregion; F17a, Upper and Middle Yarlun Zhanpo subregion. *SB*, Sichuan basin; *YP*, Yunnan plateau. Country and province abbreviations as in Fig. 1

Table 2 Average altitudes per clade of 132 samples of *Corallodiscus* included in the study

ITS clade	Average altitude (m)	SD
I-1	1756	300
I-2	2673	241
I-3	2741	449
II-1	2482	748
II-2	1285	559
II-3	2638	682
III-1	3253	550
III-2	1453	325
III-3	2503	1056
III-4	2004	565

SD standard deviation

while the remaining 54 had 1–14 base or length polymorphisms (Online Resource 5). On the basis of the type and distribution of polymorphic ITS positions, it was possible to deduce that most plants with polymorphic positions possessed nucleotide compositions of plants from the same subclades (35 cases), from between subclades of the same main clade (16) or between different main clades (3). The last category involved populations "MKK" and "QJ" and "XCL".

Divergence times

The age of the *Corallodiscus* lineage was estimated to be around 44.7 (HPD: 36.8–53.9) million years (MY), and the first split in the genus separating *C. conchifolius* from the rest 29.4 MY (HPD: 23.1–35.5). The split between Clade II and Clade III occurred about 22.6 MY (HPD: 16.4–28.4) ago. First diversifications within these clades occurred around 18.5 MY ago, with further diversification for several supported subclades (Fig. 4).

Inference of ploidy level

Chromosome counts

Plants from "Muli", "YS" and "YNL" were counted with $2n = 20$ chromosomes, while those of "LPX" and "LJB" were found to be tetraploid (Online Resource 6; Table 3).

Pollen diameter measurements and ploidy levels

The pollen diameter of plants counted as diploid ranged from 15.52 (SD: ±0.282) to 16.63 µm (SD: ±0.441) with an average of 16.07 µm (SD: ±0.402) (Table 3; Online Resource 7). The samples counted as tetraploid had pollen sizes ranging from 18.29 (SD: ±0.304) to 21.33 µm (SD: ±0.841) and an average of 20 µm (SD: ±0.978) (Table 3; Online Resource 6). Plotting the pollen diameter of all samples against their corolla length showed two clusters of pollen sizes with the counted diploid and tetraploid each residing in one cluster (Fig. 5). The average for the samples inferred as diploid (15.77 µm, SD: ±0.203, range 14.24–17.06 µm) and tetraploid (19.35 µm, SD: ±0.811, range 17.86–20.97 µm) was close to the values for the respective counted ploidy levels. Statistically, the tetraploids had a significantly larger pollen diameter compared to the diploids (*t* test, $P \leq 0.0001$), equating to an approximate 82.7% increase in volume, based on (4/3) *pi* r^3. On the basis of the chromosome counts and pollen sizes, 33 populations were inferred to represent diploids and 12 populations tetraploids (Tables 3, 4).

The relationship between pollen size and corolla length was intricate. While the pollen size varied within the respective ploidy levels only to some degree among the populations, the corolla length varied to a much greater extent, from 9.5 to 14.5 mm in diploids and 10–17 mm in tetraploids, and was thus greatly overlapping between the two ploidy levels. This might suggest that corolla size was controlled to a great extent by genetic or environmental factors, rather than ploidy level alone.

Fig. 4 BEAST chronogram with 95% HPD bars for 132 *Corallodiscus* samples based on ITS sequences. Samples shaded *yellow* and *red* were determined as diploid and tetraploid, respectively. Altitudes as means in metres (SD). Numbers along branches posterior probabilities. *Inset* below chronogram, *bar chart* of diversification events over time. HM = Hengduan Mountains; Ages: Than. = Thanetian, Bar. Bartonian, Priab. = Priabonian, Aq. = Aquitanian, Burdig. = Burdigalian, Lan. = Langhian, Se. = Serravallian, Torton. = Tortonian, M. = Messinian, Z. = Zanclean, P. = Piacenzian, G. = Gelasian, C. = Calabrian, I. = Ionian, T. = Tarantian; epochs: PA.—Palaeocene, PLI. = Pliocene, PLE. = Pleistocene, H. = Holocene; periods: PAL. = Palaeogene, Q. = Quaternary

Discussion

Phylogenetic relationships

We have assembled a comprehensive dataset in one of the largest and wide-ranging studies across China and adjacent regions to investigate mechanisms to explain the great morphological diversity found in *C. lanuginosus* with focus on the Hengduan Mountains. The phylogenetic analyses here provided the evolutionary framework and a first insight into the relationships of populations within *Corallodiscus*. It was not surprising that the *C. conchifolius* samples fell in a clade well separated from all other *Corallodiscus* samples. It is in concordance with their distinct morphology including the formation of dense mats from

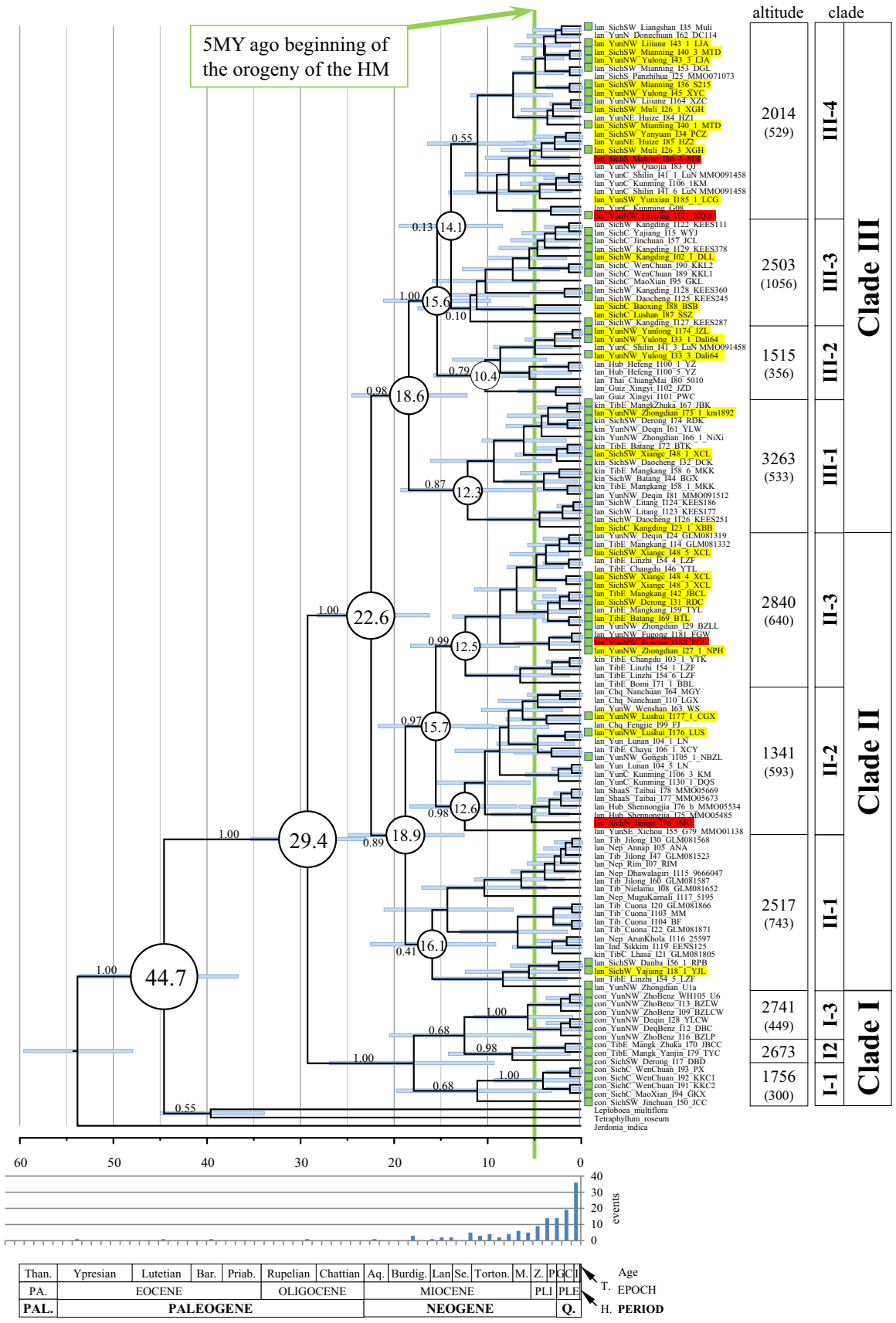

5MY ago beginning of the orogeny of the HM
altitude
clade
2014 (529)
2503 (1056)
1515 (356)
3263 (533)
2840 (640)
1341 (593)
2517 (743)
2741 (449)
2673
1756 (300)
III-4
III-3
III-2
III-1
II-3
II-2
II-1
I-3
I2
I-1
Clade III
Clade II
Clade I
44.7
29.4
22.6
18.9
18.6
16.1
15.7
15.6
14.1
12.6
12.5
12.3
10.4
60
50
40
30
20
10
0
events
Than.
Ypresian
Lutetian
Bar.
Priab.
Rupelian
Chattian
Aq.
Burdig.
Lan
Se.
Torton.
M.
Z.
PG
Cl
PA.
EOCENE
OLIGOCENE
MIOCENE
PLI
PLE
PAL.
PALEOGENE
NEOGENE
Q.
Age
T. EPOCH
H. PERIOD

Table 3 Chromosome counts, pollen diameter (±SD) and corolla length measurements for *Corallodiscus* plants from five populations

Pop. code	Locality county	Coll. no.	Chromosome number	Pollen diameter		Corolla length (mm)
				(μm)	SD	
Muli	Muli	09-1634B	20	16.36	0.441	12.0
Muli	Muli	09-1634C	20	16.32	0.378	10.0
Muli	Muli	09-1634D	20	16.07	0.500	12.0
Muli	Muli	09-1634E	20	16.63	0.563	11.0
YS	Yongsheng	12-1848_13	20	15.52	0.282	10.0
YNL	Yongsheng	12-1849_lake	20	15.95	0.543	12.0
YNL	Yongsheng	12-1849_mix	20	15.63	0.883	14.5
Average				16.07	0.402	
LJB	Yulong	09-1657B	40	20.53	0.678	14.0
LJB	Yulong	09-1657C	40	20.23	0.742	12.0
LPX	Yulong	09-1658A	40	18.29	0.304	15.5
LPX	Yulong	09-1658F	40	19.24	0.881	12.0
LPX	Yulong	09-1658a	40	21.33	0.841	15.5
LPX	Yulong	09-1658b	40	20.04	1.326	14.0
LPX	Yulong	09-1658c	40	20.35	0.726	11.0
Average				20.00	0.978	

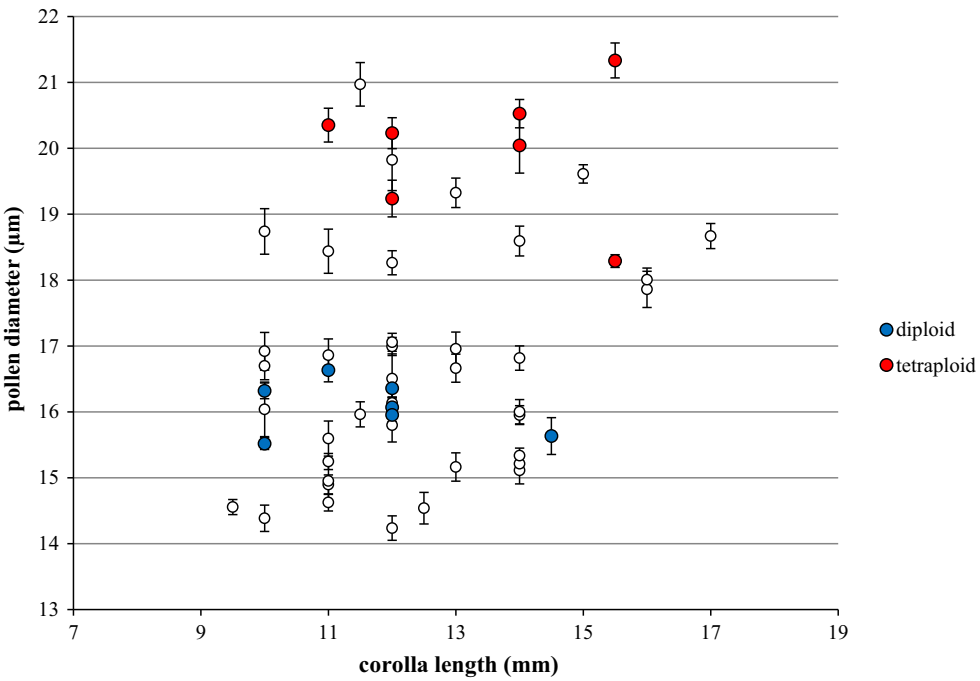


Fig. 5 Relationship between pollen diameter and corolla length in *Corallodiscus lanuginosus*. *Blue solid circles* with diploid counts ($2n = 2x = 20$); *red solid circles* with tetraploid counts ($2n = 4x = 40$). *Vertical bars* indicate (±) standard errors for pollen diameter

stolons and the septicidal capsule dehiscence, which is rare among Old World Gesneriaceae (as opposed to loculicidal in all other *Corallodiscus* taxa and most Gesneriaceae).

A more surprising result was that the *C. kingianus* samples nested among the *C. lanuginosus* samples (Figs. 2, 4), since the species had previously not been included in *C. lanuginosus* (Wang et al. 1990, 1998). The fact that not all samples of *C. kingianus* fall in one clade (Figs. 2, 4) and the inferred hybridization event of population "MKK" (Clade III-1) with a plant of Clade II-2 (Fig. 2, see also under 4.4), suggests a closer relationship with this taxon and *C. lanuginosus.* These phylogenetic findings here will be investigated in detail for their taxonomic consequences in a forthcoming separate publication.

Table 4 Pollen diameter and corolla length of 41 populations of *Corallodiscus* arranged by inferred ploidy level, diploid and tetraploid

Pop. code	Locality county	Coll. no.	Pollen diameter		Corolla length (mm)
			(μm)	SD	
Diploid					
BC	Yunlong	11-1773	16.92	0.906	10.0
BSB	Baoxing	10-1695	16.03	0.564	12.0
BTL	Batang	09-1582	15.11	0.650	14.0
CGX	Lushui	11-1777	15.97	0.443	12.0
Dali64	Dali	09-1462	14.95	0.856	11.0
DLL	Kangding	09-1607	15.34	0.363	14.0
FB	Wenxian	2010-018	17.06	0.432	12.0
GSE	Gongshan	11-1784	16.96	0.804	13.0
HYD	Yunlong	11-1796	16.86	0.784	11.0
HZ2	Huize	10-1674	16.13	0.702	12.0
JBCL	Mangkang	09-1579	16.00	0.584	14.0
JZL	Yunlong	11-1772	16.04	1.242	10.0
KM1892	Zhongdian	09-1498	14.54	0.757	12.5
LCG	Yunxian	11-1801	16.99	0.434	12.0
LJA	Lijiang	09-1465	16.66	0.673	13.0
LUS	Lushui	11-1774	15.96	0.599	11.5
MTD	Mianning	09-1622	15.25	0.387	11.0
NPH	Zhongdian	09-1489	15.95	0.449	14.0
PCZ	Yanyuan	09-1639	14.63	0.411	11.0
RDC	Derong	09-1499	14.24	0.586	12.0
S215	Yanyuan	09-1625	14.38	0.633	10.0
SSZ	Lushan	10-1689	16.70	0.659	10.0
WLP	Weixi	11-1764	14.56	0.363	9.5
XBB	Kangding	09-1589	16.82	0.586	14.0
XCL	Xiangcheng	09-1508	15.16	0.678	13.0
XGH	Muli	09-1639	14.90	0.465	11.0
XYC	Yulong	09-1469	15.80	0.804	12.0
YJL	Yajiang	09-1594	15.21	0.387	14.0
YPS	Yunlong	11-1768	15.60	0.842	11.0
YWC	Weixi	11-1761	16.50	0.841	12.0
Average			15.77	0.203	
Tetraploid					
BHZ	Mianxian	2010-023	19.33	0.706	13.0
BJE	Fugong	11-1794	20.97	1.044	11.5
DQC	Lanping	11-1766	19.61	0.440	15.0
FGL	Gongshan	11-1790	18.74	1.037	10.0
JMG	Jiange	2010-014	19.82	1.468	12.0
LJB	Yulong	09-1468	17.86	0.869	16.0
LJC	Yulong	s.n.	18.67	0.601	17.0
LYP	Lanping	11-1765	18.01	0.561	16.0
MB	Mabian	10-1681	18.26	0.577	12.0
WeiXi	Weixi	11-1763	18.44	1.061	11.0
XQ20	Lanping	11-1767	18.59	0.555	14.0
Average			19.35	0.811	

SD standard deviation

Biogeographic history

The estimated values for the stem and crown ages for *Corallodiscus* here are based on secondary calibrations points which can affect the accuracy by increased uncertainty (e.g. Schenk 2016) and in our case may seem quite high with 44.7 MY (HPD: 36.8–53.9) and 29.4 MY (HPD: 23.1–35.5). However, similar values have been obtained for the genus using multigene, multifossil and geology-calibrated trees [e.g. Petrova et al. 2015: 47.59 MY (HPD: 36.97–68.15) and 22.15 MY (HPD: 7.08–37.95); Roalson and Roberts 2016: 54.08 MY (HPD: 31.86–62.72) and 32.79 MY (HPD: 16.89–47.36)], and thus our estimates fall well within the probable. Furthermore, the HPD ranges for the crown age of *Corallodiscus* overlap greatly with those of other Gesneriaceae genera such as *Epithema* (HPD: 5.92–28.14) or *Streptocarpus* (HPD: 8.56–28.03, Petrova et al. 2015; HPD: 17.86–37.15, Roalson and Roberts 2016), and *Corallodiscus* appears to thus fall at the upper end of ages for genera in the family. Given its position on an early diverging branch of tribe Trichosporeae (Möller et al. 2009; Roalson and Roberts 2016) and huge distribution range, an old age is quite feasible. From our divergence time estimates we find that the *Corallodiscus* lineage appears to be going back as far as the Eocene epoch, a time coinciding with or just post-collision of the Indian continent with the Eurasian plate (Yin and Harrison 2000), which initiated the orogeny of the Himalayas and the QTP. The uplift of the QTP has had profound effects on the global climate, including cooler winters in northern Asia and drier winter of the Asian interior (Ruddiman and Kutzbach 1989; Shi et al. 2001). Since *Corallodiscus* species are strongly poikilohydrous, similar to the related but younger Gesneriaceae lineage including *Haberlea* (Georgieva et al. 2007; Petrova et al. 2015), this trait may have been acquired with the origin of *Corallodiscus* perhaps as an adaptation to the drier climates and habitats. The Hengduan Mountains have a strong dry season in the present day due to the rain shadow effect of the Gaoligong Shan and the Nu Shan in the West of the Three River gorges subregion (Fig. 1; E14a in Fig. 3). Although Hengduan Mountains are the centre of diversity of the genus, they cannot be the centre of origin of *Corallodiscus* since the mountains are with 5 MY too young (Tapponnier et al. 1990; Shi et al. 2001; Akciz et al. 2008), even for *C. lanuginosus* (22.6 MY), and the plants must have persisted in suitable habitats perhaps along the uprising Himalayas and QTP. Similar scenarios of origination in adjacent regions of the QTP have been observed for other plants (e.g. Wang et al. 2007; Zhang et al. 2011; Zhou et al. 2013), though not with such deep divergence as in *Corallodiscus* observed here.

The branching pattern observed in the phylogeny of *C. lanuginosus* indicates waves of diversifications reaching back as far as the mid-Miocene and peaking over the last 5 MY (Fig. 4, inset). This period could be partly linked to the orogeny of the Hengduan Mountains and the Himalayas and the intensification of the East Asian Monsoon for clades existing currently there. Other clades, such as subclade II-2 and III-2 residing today some distance from those mountains, occupy much lower altitudes (Table 2), and adaptation to elevated temperatures may be involved in their cladogenesis. It is interesting though that all subclades include members residing in the Hengduan Mountains, suggesting that these mountains were colonized repeatedly from nearby areas. Some radiations have taken place within the Hengduan Mountains themselves as indicated by subclades II-3 and III-1 (and subclades I-2 and I-3 for *C. conchifolius*) which are almost entirely composed of samples from these mountains that show divergences younger than 5 MY (Fig. 4, inset). Extensive migrations up and down mountain slopes and river valleys particularly in the Three River gorges, driven by climatic effects of glacial–interglacial cycles during the Quaternary (Li et al. 2011), may have been involved in the diversification of *C. lanuginosus* which would have created opportunities for secondary contacts, even of populations that have diverged a long time ago (e.g. subclades II and III) and now occupy the same localities.

Current distribution patterns of *Corallodiscus lanuginosus*

The diverse clades identified within *C. lanuginosus* reveal a strong geographic component (Fig. 3) and altitude ranges (Table 2). It is noticeable that the boundaries of clades within *C. lanuginosus* conform only partly with major floristic divides in China of Wu and Wu (1998). Both clades (II-2 and III-2) cross the major divide between the Sino-Japanese (region D) and Sino-Himalayan (region E) forest subkingdoms. Clade II-1 also crosses the latter boundary (region E) into the Xizang, Pamir and Kunlun region (region F) (Fig. 3). The Tanaka-Kaiyong line (Li and Li 1992, 1997), that roughly represents the Sino-Himalayan/Sino-Japanese boundary in the North but dissects the Yunnan plateau in the South, is another recognized major floristic discontinuity, but one that *Corallodiscus* clades cross (Fig. 3). This is quite in contrast to other plants whose distributions conform well to the floristic divides such as *Taxus* (Gao et al. 2007; Liu et al. 2013) or *Tsuga* (Cun and Wang 2015). These are understory forest trees, unlike *Corallodiscus* which grows rocky slopes or cliffs often in the open or along forest margins and thus has different ecological requirements allowing their spread not to be restricted by the requirement of the presence of forests. Their seeds are small about 0.7 mm long (Beaufort-Murphy 1983), and the inflorescences present the capsules

for short-distance wind dispersal of the seeds, allowing a relatively rapid habitat expansion in the relatively open habitats the plants occupy.

Accumulation of diversity in the Hengduan Mountains region

The highest genetic diversity of extant *Corallodiscus* was observed in the Hengduan Mountains region (region E14). This is an area recognized for its high levels of biodiversity (e.g. Li and Li 1993; Myers et al. 2000). The Three Rivers gorges subregion (E14a) is particularly rich in both taxonomic and genetic diversity with members of six subclades, out of seven of the two *C. lanuginosus* clades (plus two of three subclades of *C. conchifolius*), present there (Fig. 3). Such dense sympatricism appears to have had consequences for the evolution of the plants. We found evidence for repeated historical and contemporary hybridization between populations. In some cases, these involved neighbouring populations of the same clade (Fig. 4; Online Resource 5). For others, crosses between the two main clades II and III were inferred from the distribution of ITS polymorphisms (e.g. MKK, QJ, XCL). In two cases, the presence of plants with ITS types from different major clades (II and III) was observed in the same population (i.e. KM, XCL). For most of these cases, not surprisingly, the populations involved in the genetic exchanges came from areas where the respective clades overlap (Fig. 3).

The consequences of secondary contacts between populations and species depend on their genetic distance (e.g. Stebbins 1959; Rieseberg and Wendel 1993). Recently evolved taxa retain a high degree of fertility in hybrid offspring, and this can result in homoploid speciation, as observed for other Gesneriaceae such as *Streptocarpus* (Hilliard and Burtt 1971) or the Betulaceae genus *Ostryopsis* (Liu et al. 2014). With increasing genetic distances or time of allopatry, lineages diverge and their hybrid offspring become increasingly sterile upon secondary contacts. For example, the estimated divergence time for the parents of the hybrid species *Oreocharis* × *heterandra* is estimated with ca 6 MY and the plants are completely sterile (Puglisi et al. 2011). Our main clades are much older, with a stem age of ca 22.6 MY. Secondary contact and hybridization between the two main lineages of *Corallodiscus* will likely produce sterile F1 plants and should evolutionarily be a dead end, except where allopolyploids are formed that restores fertility. This also creates reproductive barriers with their diploid parents instantaneously and permanently, since polyploidy is biologically virtually irreversible (Meyers and Levin 2006). We determined and inferred the existence of several tetraploid populations, occurring in the Three River gorges subregion and around the Sichuan basin which may be an indication of past hybridization events. Particularly, the former region included six populations with inferred polyploids four of which possessed ITS regions too polymorphic to be sequenced by direct sequencing (triangles in Fig. 3). The phylogeny (together with the distribution of diploids) suggests that such tetraploidization events occurred several times independently in *C. lanuginosus*, something akin to *Tragopogon* (Soltis et al. 2004), though here the secondary contact had an anthropogenic background due to the introduction of several species in the same habitat. In *Corallodiscus*, however, this was likely driven by oscillating distribution ranges. Climate-driven changes in distributions can shift species boundaries or results in local adaptations (e.g. Comes and Kadereit 1998; Hewitt 2004; Liu et al. 2013). These stationary, locally adapted entities are candidates for mixing with entities "passing through" during climate cycles and potential scenarios for secondary contacts ensue.

Such secondary contact—hybridization—polyploidization scenarios would increase biological richness and may have occurred in the Hengduan Mountains, since polyploidy is known to exist among and within congeners here, though perhaps relatively infrequently since the overall levels and importance of polyploidy are thought to be low in this region (Nie et al. 2005; Yuan and Yang 2008), with an exception perhaps being *Anaphalis*, where only two species out of 12 were diploid, seven tetraploid and one showed a range of polyploidy levels (Meng et al. 2014). This relative high abundance of polyploidy is similar to that found in *Corallodiscus* where for more than a quarter of populations analysed tetraploidy was inferred (Table 4). None of the previous studies place the cytological results in a phylogenetic context. Here we provide the time frame, phylogeographic and cytological data for such repeated secondary contact—hybridization—polyploidization scenario. The consequence of such processes may have resulted in the patchy distribution of species as defined in the past in the genus *Corallodiscus* (Burtt 1947; Wang et al. 1990). This is perhaps conflagrated by the continuous variation of morphological characters that seems to be a combination of genetic as well as ploidy effects, as illustrated by corolla size (Fig. 5), which ultimately lead to the reduction in taxonomic units in the genus and the establishment of the variable *C. lanuginosus* (Wang et al. 1998).

Conclusions

The genus *Corallodiscus* represents a surprisingly old lineage with an origin in the mid-Eocene from within *C. lanuginosus* split in the Oligocene and steadily diversified with an acceleration in the last five million years which

coincides with the orogeny of the Hengduan Mountains. Pre- and post-Quaternary climate oscillation-driven expansions and contractions of distribution ranges coupled with the acquisition of high-temperature tolerance allowed the spread of the plants to lower altitudes south of the Sichuan basin. The expansion contraction cycles allowed allopatric divergence but also extensive hybridization on secondary contact to occur. We further found repeated cases of tetraploidization events to have taken place, instantly creating reproductive barriers that allowed local accumulation of biodiversity. Secondary contacts, hybridization and introgression appear to be responsible for the high variation and complex distribution of morphological diversity in *C. lanuginosus*. Polyploidization as a reinforcing genetic barrier added to the diversity in space and time particularly in the Hengduan Mountains. While allopatric diversification and ecological adaptation have been proposed as mechanisms underlying the high diversity in this area, our study here adds hybridization and polyploidization as further factors.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement The authors comply with all rules of the journal following the COPE guidelines; all authors have contributed and approved the final manuscript.

Information on Electronic Supplementary Material

Online Resource 1. List of samples of *Corallodiscus* included in the phylogenetic analysis, plus three outgroup samples, with collector and collection information, population codes and GenBank accession number.
Online Resource 2. Diagnostics of the Bayesian inference analysis of 132 *Corallodiscus* samples plus three outgroup samples based on ITS sequence data. (a) Bayesian run statistics; (b) plot of generations versus *Ln*L values for run 1; (c) plot of generations versus *Ln*L values for run 2; (d) posterior probabilities of run 1 versus run 2; (e) symmetric topological tree differences within and between MCMC run 1 versus run 2; (f) plot of splits 1–20 sorted by widest range for run 1; (g) plot of splits 1–20 sorted by widest range for run 2. b–g obtained using AWTY (Wilgenbusch et al. 2004).
Online Resource 3. List of samples of *Corallodiscus lanuginosus* cytologically investigated, with collector and collection information, and population codes.
Online Resource 4. List of samples of *Corallodiscus lanuginosus* palynologically investigated, with collector and collection information, and population codes.
Online Resource 5. Distribution of ITS base polymorphisms and inferred crosses among samples of *Corallodiscus lanuginosus* (lan) and *C. kingianus* (kin).
Online Resource 6. Chromosome counts of *Corallodiscus lanuginosus* plants with (a) $2n = 20$ chromosomes (2013-28-F2-1e) and (b) $2n = 40$ chromosomes (2013-03-F3-1i).
Online Resource 7. Pollen measurements of *Corallodiscus lanuginosus* plants with (a) diploid (PCZ) and (b) tetraploid (JMG) chromosome complements.

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