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ORIGINAL ARTICLE

Species evolution and quality evaluation of four *Coptis* herbal medicinal materials in Southwest China

Hanting Chen[1] · Gang Fan[1] · Yang He[1]

Abstract *Coptis* herbs, which are important herbal medicinal materials, are the dried rhizomes of various plants. In China's herbal market, drying herbs can severely influence morphological markers such as shape, color, and odor, which make consumers difficult to precisely identify the herbs and effectively evaluate the quality. Here, we present the phylogenetic analysis of four *Coptis* herbal medicinal materials based on internal transcribed spacer sequences. *C. chinensis*, *C. omeiensis*, *C. deltoidea* and *C. teeta* constituted a monophyletic group. In this group, *C. omeiensis* and *C. deltoidea* cluster together, and they form the sister group with *C. teeta*, and *C. chinensis* locate the outermost of *C. omeiensis*, *C. deltoidea* and *C. teeta*. At the same time, the HPLC–DAD method was applied to simultaneously analyze main alkaloids from seventeen different samples. On the basis of the robust phylogenetic topology, the parsimony reconstructions of six effective medicinal constituents were implemented to elucidate the evolutionary history in *Coptis* herbs, and thus evaluate the herbal quality. The results showed that *C. chinensis* had been considered to be the best herb, not only the high content in single constituent but also in total alkaloids. In addition, all the samples from seventeen habitats were considered as qualified herbs, and they could meet the requirements of national quality standards for *Coptis* herbs in China.

Keywords *Coptis* herbs · Internal transcribed spacer (ITS) · Evolution · Alkaloid · Herbal quality

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Introduction

Coptis herbs, as famous Traditional Chinese Medicine, have been used for prevention and treatment of human diseases, such as cancer and inflammation for centuries (He et al. 2015). *Coptis* herbs can clear away heat and drying dampness in the treatment of diarrhea, dysentery, jaundice, hematemesis, carbuncles, and abscesses. They also purge the sthenic fire and clear away toxic material in cases of seasonal febrile disease, carbuncles, sore throats, etc. (The Pharmacopoeia of the People's Republic of China 2015; Liu et al. 2006). According to the Pharmacopoeia of the People's Republic of China (2015 edition), *Coptis* herbs are the dried rhizomes of ranunculaceous plants including *Coptis chinensis* Franch., *Coptis deltoidea* C.Y. Cheng et Hsiao, and *Coptis teeta* Wall (Qiao et al. 2009), and these three species are all distributed and endemic in Southwest China (Teng and Choi 2012), especially in Sichuan, Chongqing, Yunnan, and Hubei. Concurrently, the *Coptis omeiensis* C.Y. Cheng was always used as the substitute because of their similar medicinal constituents. Traditional means of herbal species classification or taxonomy mainly rely on the inspection of morphological markers such as shape, color, texture, and odor (Heinrich 2007). However, the morphological markers were destroyed by dry herbs, and thus, it became difficult to classify the different species and evaluate the quality even for the specialists (Song et al. 2009). So it is imperative to find new ways and/or methods to classify the *Coptis* herbs from different sources and to evaluate the herbal quality.

A number of research groups have used molecular systematics methods to reconstruct plant systematics and solve systematic problems, as it is a difficult problem in previous taxonomic studies (Hörandl et al. 2005; Lane et al. 2007;

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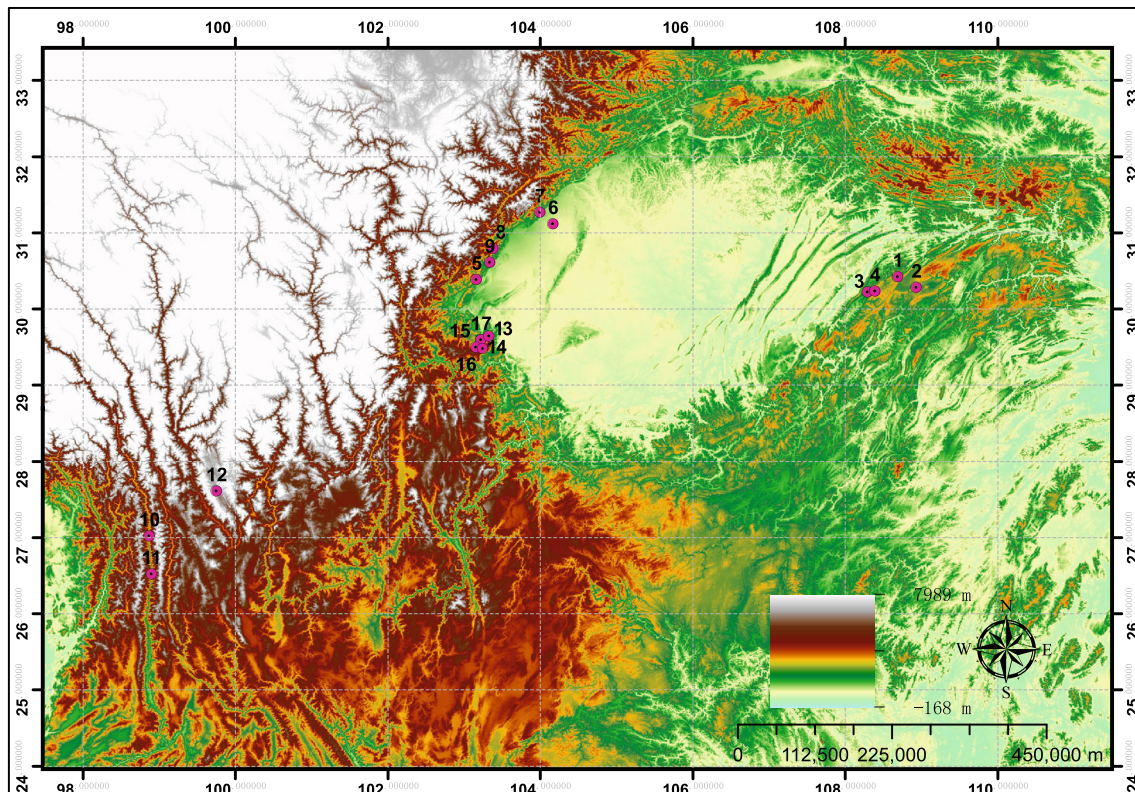
Fig. 1 Sampling of the four *Coptis* species. Map showing the sampling sites of the here included specimens

Yassin et al. 2010). To date, the molecular phylogeny of genus *Coptis* has been reported (He et al. 2014; Xiang et al. 2016), but too little attention was paid to the medicinal plants of genus *Coptis*. In Southwest China, various climatic zones and different soil types provided a wide range of environmental conditions for the growth with profoundly different vegetation types (Zhang et al. 2004), and environmental factors possibly produced conservation of genetic mutations (He et al. 2014). So, molecular phylogenetic methods could be used to fully resolve the species taxonomy of *Coptis* herbs.

Phytochemical and pharmacological studies on medicinal plants revealed that *Coptis* herbs contained a number of alkaloids, such as berberine, palmatine, jatrorrhizine, coptisine, columbamine, and epiberberine (Kuang 2000; Qiao et al. 2009). Since application of *Coptis* herbs is growing steadily, development of a useful and suitable method for their quality control was urgently required (Kong et al. 2009). Berberine had been usually used as a marker to monitor the drug quality in the official Chinese Pharmacopoeia. Comparing with a single berberine, the assay for a set of main alkaloids is an effective way to evaluate the quality of botanical drugs. Thus, the qualitative and quantitative analyses of several high-content constituents in *Coptis* herbal extracts will help us better evaluate and regulate the drug quality.

Methods

Medicinal plant materials and sequencing

Samples were collected from seventeen different habitats in Southwest China (Fig. 1). Leave and rhizome samples were separated from different plant sources. Microquantities of DNA were isolated directly from dried specimens using a modified CTAB method (Doyle and Doyle 1987). Amplifications of the nuclear ITS regions were carried out using the universal primer set, forward primer (5′-AACAAGGTTTCCGTAGGTGA-3′), and reverse primer (5′-TATGCTTAAA YTCAGCGGGT-3′) (Nickrent et al. 1994). The PCR was conducted as follows: denaturation at 94 °C for 4 min, 30 cycles at 94 °C for 30 s, 45 °C for 30 s, and 72 °C for 2 min, and then 72 °C for 5 min (Schuettpelz et al. 2002). PCR products were sent to Sangon Biotech (Shanghai, China) Co., Ltd., for sequencing. And the sequencing results were submitted to NCBI (Accession numbers: KX983995-KX984011).

Preparation of sample solution

All the tested samples were crushed into powder and dried at 50 °C for 24 h before use. The dried powders (approximately 100 mg) were accurately weighed into a clean

Erlenmeyer flask, and extracted with 50 mL of hydrochloric acid–methanol solution (1:100, v/v) by ultrasonication for 30 min. After filtration, the filtrate was adjusted to the original weight with the extraction solvent. The sample solutions were filtered through a 0.45-µm membrane prior to an injection into the HPLC system. The concentrations of berberine, palmatine, coptisine, epiberberine, columbamine, and jatrorrhizine in each sample were determined using the established calibration curves.

Apparatus and chromatographic conditions

HPLC analysis was performed on an Agilent 1200 Series LC system (Agilent Technologies, Santa Clara, CA, USA) equipped with an auto-sampler, a quaternary pump, a diode array detector (DAD), and a column thermostat. The chromatographic separation was carried out on a Xtimate C18 HPLC column (4.6 mm × 250 mm, 5 µm), and maintained at 30 °C. The detection wavelength was set at 270 nm. The mobile phase was acetonitrile (A) and 30 mmol/L ammonium bicarbonate buffer containing 0.7% (v/v) ammoniae aqua and 0.1% (v/v) triethylamine (B) with a gradient elution program of 10–25% A at 0–15 min, 25–30% A at 15–25 min, and 30–45% A at 25–40 min. All injection sample volumes were 10 µL.

Phylogenetic analysis

The successfully sequenced genes were initiated a complete alignment by Clustal X 1.81 (Thompson et al. 1997) with default parameters, and then the result was repeatedly checked by eye to ensure its accuracy. After the nucleotide substitution model within each gene was tested by software jModeltest 2.1.4 (Darriba et al. 2012), the best evolutionary model was selected by Akaike information criterion (AIC).

According to the best evolutionary model selection, the whole sequence was, respectively, constructed with maximum likelihood (ML) and Bayesian inference (BI). ML with RAxML 8.0.17 (Stamatakis 2014) was performed using a GTR+G model of sequence evolution for the final likelihood search and switched to the per-site rate category model during the fast bootstrapping with 1000 runs. BI was conducted using MrBayes 3.2.2 (Ronquist and Huelsenbeck 2003), while the evolutionary model was used according to the result of jModeltest, and we ran four concurrent Markov chain Monte Carlo (MCMC) calculations of five million generations while sampling once every 100 generations. The first 25% of sampled trees were discarded as burnin and we used the remaining samples to generate a majority-rule consensus tree. We repeated all MCMC runs twice to confirm the consistent approximation of the posterior parameter distributions.

For understanding the evolutionary changes of effective constituents in our selected four species, the parsimony reconstruction was used to estimate the tendency of variation, which was implemented in Mesquite 3.0.4 (Maddison and Maddison 2009).

Results

Geographic character of *Coptis* herbs

The seventeen samples were successfully collected from the main habitats. Figure 1 indicates that the *Coptis* herbs were mainly distributed at the edge of Sichuan Basin and Yunnan Province. The *Coptis* herbs were mainly grown in 500–2000 m above sea level in the mountain forest or in the valley shade. Because of similar environmental conditions for the growth, the *Coptis* herbs have the similar quality in different species. Moreover, they are all distributed and endemic in Southwest China, and almost all wild plants of *Coptis* herbs are endangered medicinal herbs.

### Sequencing and molecular evolution analyses

Seventeen ITS sequences were successfully obtained from the genomic DNA of different samples by the specific primer set. The efficiency of ITS amplification from *C. chinensis* and *C. omeiensis* reached up to 100%; however, *C. deltoidea* and *C. teeta* were 92 and 90%, respectively.

After alignment, the seventeen ITS sequences produced about 700 nucleotide sites. The best evolutionary model was selected as GTR+G model according to the Akaike's information criterion (AIC) (Akaike 1973). The topology produced from maximum likelihood (ML) and Bayesian inference (BI) was coincident, and all currently recognized species were monophyletic. In general, *C. chinensis*, *C. omeiensis*, *C. deltoidea* and *C. teeta* constituted a monophyletic group. In this group, *C. omeiensis* and *C. deltoidea* cluster together, and they form the sister group with *C. teeta*, and *C. chinensis* locate the outermost of *C. omeiensis*, *C. deltoidea* and *C. teeta* (Fig. 2). The *Coptis* herbs in Southwest China can be successfully classified by the ITS sequences.

Chemical analysis of main alkaloids

The proposed HPLC–DAD method was applied to simultaneously analyze main alkaloids from seventeen different samples. The main six peaks were identified, i.e., berberine, palmatine, coptisine, epiberberine, columbamine, and jatrorrhizine, and subsequently evaluated by comparison with authentic standards (Table 1). *C. chinensis*, as the most important species for daily use in clinical medication, contain the obviously different contents not only in total

Fig. 2 Bayesian inference tree derived from the part of ITS genes. *Numbers above the lines or besides the nodes are* Bayesian posterior probabilities and maximum likelihood analyses (1000 replicates)
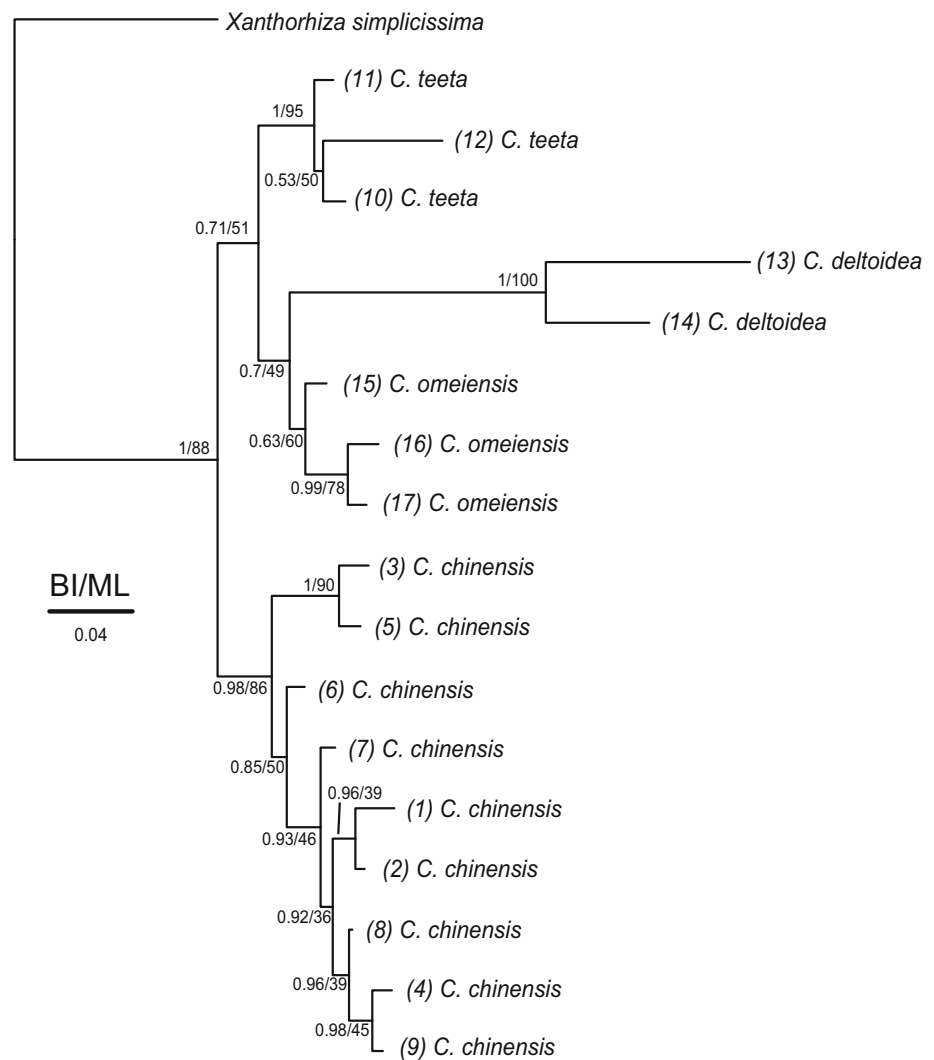
alkaloids but also in six main alkaloids compared with *C. teeta*, *C. omeiensis* and *C. deltoidea*. From Table 1, we can find that the content of jatrorrhizine in *C. deltoidea* and *C. teeta* is marginally higher than that in *C. chinensis* or *C. omeiensis*. Especially in epiberberine, *C. teeta*, *C. omeiensis*, and *C. deltoidea* have lower contents than *C. chinensis*, but it was not detected in *C. omeiensis*. When it comes to other contents, *C. chinensis* has far higher than that in *C. teeta*, *C. omeiensis,* or *C. deltoidea*.

The quality evaluation of *Coptis* herbs

The six indexes of effective medicinal constituents (jatrorrhizine, columbamine, epiberberine, coptisine, palmatine, berberine) were successfully achieved corresponding to each sequenced specimen. According to the confirmed phylogenetic topology from ML and BI, the parsimony reconstructions about the six effective medicinal constituents were implemented. The evolutionary

changes of effective medicinal constituents are shown in Fig. 3. From the ancestor node, we can find that the content of each effective medicinal constituent is different, the content in *C. chinensis* is almost higher, and other three species are lower than that of ancestor node. At the same time, Fig. 3 shows that there were some differences in the content in regard to the different intraspecific habitats. These results provided us with the possibility to evaluate the quality of *Coptis* herbs.

Discussion

The distribution and evolution of *Coptis* herbs

Seventeen samples of *Coptis* herbs were collected to reconstruct the phylogeny for species evolution by ITS sequences. Originating from the common ancestor, as a result of the differences in growth environment, late

Table 1 Contents of six main alkaloids of four *Coptis* herbal medicinal materials from 17 different habits (mg/g)

No.	Species	Source	Jatrorrhizine	Columbamine	Epiberberine	Coptisine	Palmatine	Berberine	Total
1	*Coptis chinensis* Franch	Moudao village, Lichuan city, Hubei province	4.66 ± 0.42	4.37 ± 0.34	15.46 ± 0.86	25.34 ± 0.54	16.76 ± 0.75	80.34 ± 0.95	146.89 ± 3.54
2		Yulong village, Lichuan city, Hubei province	5.23 ± 0.27	6.76 ± 0.15	16.87 ± 1.54	26.34 ± 1.21	20.36 ± 1.44	81.22 ± 1.11	156.78 ± 2.63
3		Yuelai village, Shizhu county, Chongqing city	3.43 ± 0.36	5.34 ± 0.21	13.44 ± 0.67	27.53 ± 0.98	16.64 ± 0.82	75.46 ± 1.36	141.84 ± 2.35
4		Huangshui village, Shizhu county, Chongqing city	4.22 ± 0.26	4.74 ± 0.17	15.71 ± 0.76	25.67 ± 0.84	16.46 ± 0.72	76.81 ± 1.26	143.63 ± 3.63
5		Nanbao village, Qionglai city, Sichuan province	5.45 ± 0.64	6.12 ± 0.24	16.76 ± 1.27	23.23 ± 0.63	21.76 ± 1.26	73.87 ± 0.88	147.19 ± 4.23
6		Wazhai village, Shifang city, Sichuan province	4.65 ± 0.25	3.86 ± 0.07	15.87 ± 0.65	26.36 ± 1.47	18.76 ± 1.14	85.23 ± 1.36	154.73 ± 3.26
7		Bajiao village, Shifang city, Sichuan province	4.07 ± 0.22	4.14 ± 0.08	11.60 ± 0.58	19.83 ± 0.75	18.48 ± 1.43	66.10 ± 1.11	124.23 ± 2.87
8		Lanniba village, Chongzhou city, Sichuan province	3.34 ± 0.28	3.76 ± 0.42	12.35 ± 1.22	26.67 ± 0.56	16.88 ± 0.85	75.35 ± 0.95	138.35 ± 3.83
9		Xieyuan village, Dayi country, Sichuan province	6.45 ± 0.15	8.78 ± 0.57	17.50 ± 0.37	27.35 ± 1.55	26.54 ± 0.45	88.86 ± 1.82	175.48 ± 2.64
10	*Coptis teeta* Wall	Lumadeng village, Fugong county, Yunnan province	6.38 ± 0.21	1.58 ± 0.65	0.46 ± 0.10	15.07 ± 0.78	5.23 ± 0.75	83.67 ± 0.97	112.42 ± 3.13
11		Pihe village, Fugong county, Yunnan province	6.07 ± 0.09	1.73 ± 0.23	0.36 ± 0.07	14.93 ± 0.37	4.61 ± 0.58	78.99 ± 1.58	106.71 ± 3.03
12		Zhongdian country, Diqing city, Yunnan province	7.76 ± 0.28	1.58 ± 0.37	0.66 ± 0.11	17.81 ± 1.61	5.24 ± 0.98	84.85 ± 1.74	117.93 ± 3.95
13	*Coptis deltoidea* C. Y. Chenag et Hsiao	Heishan village, Hongya county, Sichuan province	7.27 ± 0.16	2.15 ± 0.17	2.73 ± 0.15	11.82 ± 0.71	5.88 ± 0.55	47.06 ± 0.66	76.93 ± 2.75
14		Heilin village, Hongya county, Sichuan province	8.81 ± 0.21	1.80 ± 0.14	1.90 ± 0.22	12.18 ± 1.25	6.77 ± 0.77	48.37 ± 1.13	79.85 ± 3.64
15	*Coptis omeiensis* C.Y. Cheng	Heishan village, Hongya county, Sichuan province	4.43 ± 0.17	3.17 ± 0.06	0.00	21.23 ± 0.66	9.14 ± 0.52	63.29 ± 1.76	101.22 ± 2.27
16		Heilin village, Hongya county, Sichuan province	4.72 ± 0.28	2.85 ± 0.31	0.00	16.68 ± 0.42	9.43 ± 0.96	64.72 ± 0.47	98.40 ± 2.26
17		Gaomiao village, Hongya county, Sichuan province	4.97 ± 0.13	2.30 ± 0.75	0.00	17.39 ± 1.36	8.50 ± 0.86	56.39 ± 1.23	89.55 ± 3.56
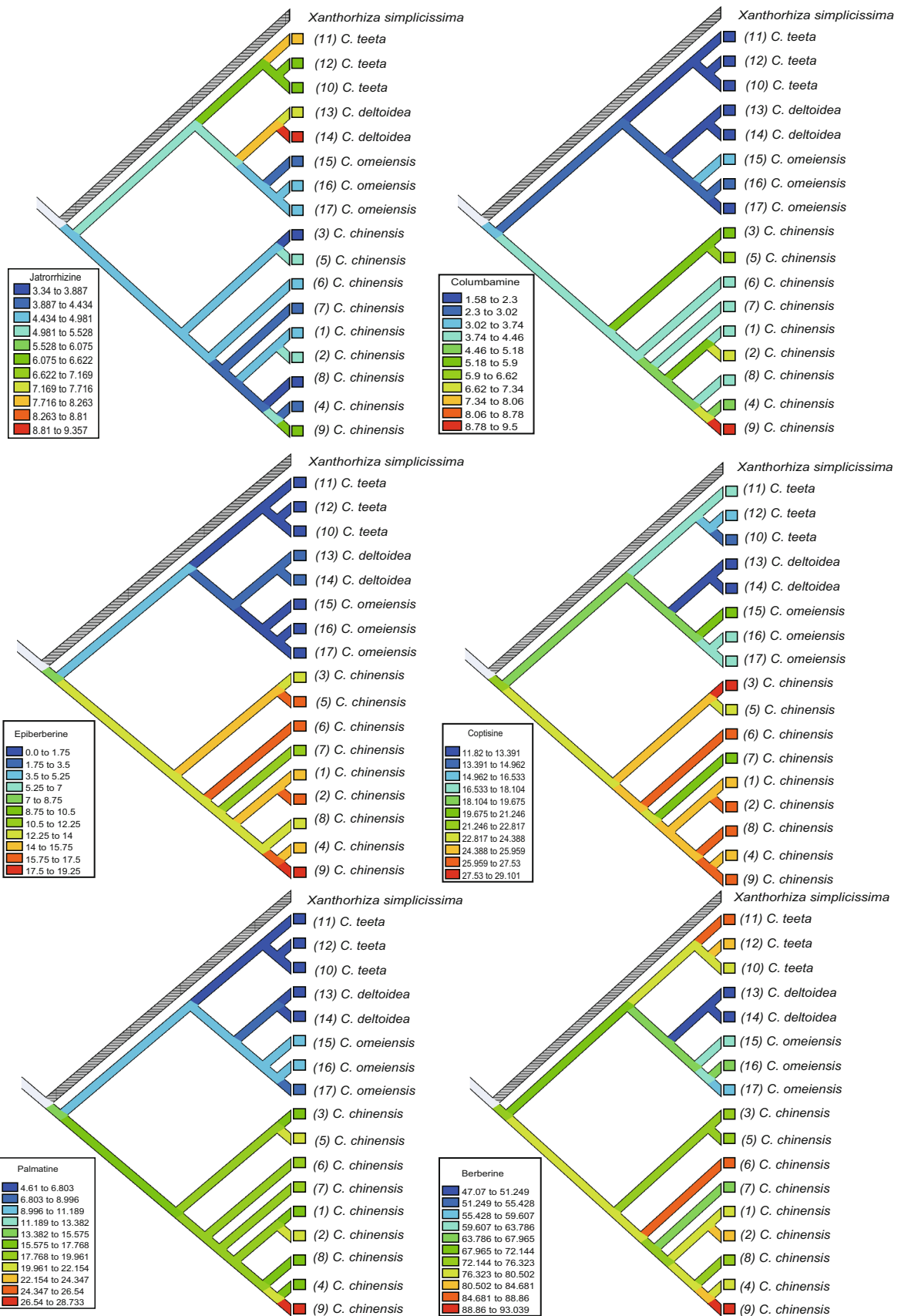
◀ **Fig. 3** Parsimony reconstruction of six selected effective components (Jatrorrhizine, Columbamine, Epiberberine, Coptisine, Palmatine, Berberine). The topology of phylogenetic tree was used from the result of Bayesian inference

evolution and herbal quality may result in large differences in *Coptis* herbs. As shown in Fig. 2, *C. teeta*, *C. deltoidea,* and *C. omeiensis* cluster as one group, and this group is sister to *C. chinensis*. The results indicated that the *Coptis* herbs from different species can be successfully classified by phylogenetic analyses.

Previous studies have shown that *C. deltoidea* and *C. omeiensis* have the most close genetic distance in the medicinal material in *Coptis* (He et al. 2014; Xiang et al. 2016), also as shown in Fig. 2. This result may be attributed to their closely geographic intercept (Fig. 1), and the two species are mainly distributed on both sides of Mount Emei in Sichuan province (Hu et al. 2011); the uplift of Mount Emei may cause the biotic geographic isolation, leading to the speciation of *C. deltoidea* and *C. omeiensis*. The *C. teeta* is grown only in Yunnan province, and it has more special growth environment than other three species. The *C. chinensis* has the most extensive distribution and growth quantity in *R. coptidis*, and we collected the samples from Sichuan, Chongqing, and Hubei (Wagner et al. 2011). Different growth circumstances caused differential genetic relationship in *C. chinensis* and gave rise to difference in constituents and contents of alkaloids, thereby resulting in different attributes in drug quality.

Chemical analysis of *Coptis* herbs

Coptis herbs are commonly used herbal drugs in TCM, and the detection of chemical constituents has been reported by many researchers (Chen et al. 2008; Li et al. 2009). The main active constituents of *Coptis* herbs are protoberberine alkaloids, which are also important marker constituents in the quality evaluation of *Coptis* herbs (Kuang 2000; Kong et al. 2009). Therefore, in this study, the six main protoberberine alkaloids (i.e., berberine, coptisine, palmatine, epiberberine, columbamine, and jatrorrhizine) were determined simultaneously in *Coptis* herbs by conventional HPLC method. It was found that the six alkaloids were detected in almost all samples of *Coptis* herbs with the exception of epiberberine, which was absent in three *Coptis omeiensis* samples. Compared to other constituents, the berberine content always showed the highest in all the samples, as previously reported (Qiao et al. 2009). On the other hand, the content of total alkaloids ranged from 76.93 to 175.48 mg/g, showing a variation of about twofold in the four species, which indicated that the contents of the six active alkaloids were

quite different according to their origins. Moreover, *C. chinensis* has the higher content of total alkaloids than those in *C. teeta*, *C. omeiensis* and *C. deltoidea*. This is also the reason why the *C. chinensis* can be acted as one of the main *Coptis* herbs for cultivation and application.

The evolution and quality evaluation of *Coptis* herbs

In order to elucidate the evolutionary relationship of *Coptis* herbs, especially derived from the content of alkaloids, more attention should be paid. Parsimony reconstructions of the six selected effective constituents (jatrorrhizine, columbamine, epiberberine, coptisine, palmatine, and berberine) herein were implemented and no previous studies had been focused on this aspect. The topology of phylogenetic tree was used from the result of Bayesian inference. From these six constituents, they might have the same evolutionary trends and the ancestor node that is located between the content of *C. chinensis* and other three species (i.e., *C. teeta*, *C. omeiensis*, and *C. Deltoidea*) has the same evolutionary direction, but *C. chinensis* has the opposite direction for evolution. The inconsistency in the direction of evolution caused differences not only in the content of effective constituents but also in the herbal quality.

In the official Chinese Pharmacopoeia, berberine alone was assigned as the marker composition for the quality evaluation of *Coptis* herbs (The Pharmacopoeia of the People's Republic of China 2015). However, in general practices, assessment of multiple constituents is objective and reasonable than a constituent in quality evaluation of *Coptis* herbs (Kong et al. 2009). Moreover, simultaneous determination of major bioactive constituents is also necessary in the practice of quality control. In this work, *C. chinensis* has been considered to be the best herb among *Coptis* herbs, because it has the high content not only in single constituent but also in total alkaloids. All the samples from seventeen habitats were recognized as qualified herbs, and they could meet the requirements of national quality standards in China. Due to different growth environments, different habitats of *C. chinensis* have different herbal qualities. The samples from Dayi have the highest quality, which has the highest content in single constituent and high content of total alkaloids in all samples, compared to other samples.

Compliance with ethical standards

Conflict of interest The author declares no conflict of interest.

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