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宽翅菘蓝系统发育关系和分类地位研究

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摘　要:宽翅菘蓝(*Isatis violascens*)是分布于新疆准噶尔盆地的一种早春短命植物。基于形态的相似性,有人建议将宽翅菘蓝作为 *Isatis emarginata* 同义体。目前宽翅菘蓝尚未得到分子水平的研究,其系统发育地位也不清楚。该研究对宽翅菘蓝的ITS区进行测序,结合GenBank数据库中菘蓝属物种的ITS序列对宽翅菘蓝的系统关系和分类地位进行研究。最大简约法,最大似然法和贝叶斯法3种方法分析表明:菘蓝属物种聚为两个分支;宽翅菘蓝与 *I. emarginata* 和小果菘蓝具有较近的亲缘关系;基于形态相似性和较近的遗传距离,支持将宽翅菘蓝与 *I. emarginata* 合并为一个物种,并把宽翅菘蓝作为 *I. emarginata* 的同义种。

关键词:宽翅菘蓝;系统发育;ITS;合并

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Phylogenetic Relationship and Taxonomic Status of *Isatis violascens* Bunge(*Isatis*, Brassicaceae)

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Abstract: *Isatis violascens* Bunge is a spring ephemeral plant occurred in the sandy deserts of Junggar (or Dzungaria) Basin. It was suggested as a synonym of *Isatis emarginata* based on their morphology similarity. *I. violascens* has not been included in any molecular studies to date and its phylogenetic relationship is unknown. In present study, the nuclear ribosomal internal transcribed spacer(ITS) of *I. violascens* was sequenced. 27 samples representing 18 *Isatis* species were analyzed using most parsimony, most likelihood and Bayesian method. Two well supported clades of *Isatis* were confirmed. *I. violascens* was closely related with *I. emarginata* and *I. minima*. From the evidence of similarities of morphology and low distance of ITS sequences, we provisionally agree with the merge of the two species and the reduction of *I. violascens* to synonym of *I. emarginata*.

Key words: *Isatis violascens*; phylogenetic relationship; ITS; merge

Isatis L. comprises of approximately 79 species[1] and nearly 90% of its members are distributed in Irano-Turanian region[2-3]. Based on morphology characters, Al-Shehbaz[4] suggested that the genus *Isatis* and genera *Pachypterygium*, *Sameraria*, *Boreava*, *Spirorrhynchus*, and *Tauscheria* should be included in tribe

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Isatideae. Molecular phylogenetic studies indicated a close relationship of these genera[5-7] and supported the inclusion of *Isatis*, *Pachypterygium*, *Boreava* and *Tauscheria* in tribe Isatideae. The tribe is characterized by having indehiscent, 1-or rarely 2-seeded angustiseptate fruits, yellow or rarely whitish flowers, sessile and often auriculatecauline leaves, and simple or no trichomes[2,4].

Convergence is quite wide spread in almost every morphological character in the Brassicaceae[4,8-11]. Therefore, the phylogenetic relationships based solely on morphology easily lead to erroneous conclusions[4,12-13]. Fruit morphology structures provide the most diagnostic characters in the genus *Isatis* and are essential for the reliable determination of species[3,14-15]. However, *Isatis* species are sometimes highly morphological polymorphic, even in fruits structure (e. g. *I. cappadocica*) and make the limits of *Isatis* still controversial. With critical studies, it is likely that the total number of species be reduced to a little over 50[4].

DNA barcoding is a new biological tool to achieve accurate, rapid species identification by using short DNA regions[16-17]. Combining DNA barcode sequences with morphological characters can fasten the rate of identification and classification of species[18-21]. The nuclear ribosomal internal transcribed spacer(ITS) was suggested to be as complementary plant barcoding regions at the Third International Barcoding of Life Conference[22] and is currently the most widely used marker in phylogenetic analyses of the Brassicaceae[5,7,10,12,23-26].

I. violascens is a spring ephemeral plant distributed in Iran, Afghanistan, Pakistan and Turcomania. In China, it occurres mainly in the Gurbantunggut Desert of the Junggar (or Dzungaria) Basin in Xinjiang. Based on morphology *I. violascens* was suggested as a synonym of *I. emarginata* in Flora of Pakistan but not followed in Flora of China. Until now, *I. violascens* has not been included in any molecular studies. Its phylogeny position in the genus *Isatis* is unknown. The aim of the present study is to sequence the barcode region of ITS from *I. violascens*, investigate it's phylogenetic position within *Isatis* and test its taxonomic status.

1　Materials and methods

1.1　Taxa collection

A total of 27 *Isatis* ITS sequences were included both retrieved from Genbank and newly sequenced. *Aethionema* was chosen as outgroup because it had been demonstrated sister to all other Brassicaceae[27-29]. Detailed sequences information, geographic origin and GenBank accession numbers are provided in Table 1.

1.2　DNA extraction, PCR amplification and sequencing

Total DNA was extracted by CTAB protocol[30]. PCR amplification of ITS were performed in a volume of 50 μL, containing 0.2 mmol/L dNTPs, 0.5 μmol/L

Table 1　Origin, citation and GenBank accession numbers of taxon used in the current study

	Taxon	Geographic origin(Country)	Genbank accession No.
1	*I. brevipes*	n. a.	GQ424551
2	*I. brevipes*	Iran: Kerman	GQ131326
3	*I. buschiana*	Iran: Azarbaijan	GQ131310
4	*I. cappadocica* subsp. *besseri*	Iran: Ardebil	GQ131311
5	*I. cappadocica* subsp. *cappadocica*	Iran: Azarbaijan	GQ131312
6	*I. cappadocica* subsp. *macrocarpa*	Iran: Kordistan	GQ131333
7	*I. cappadocica* subsp. *stenophylla*	Iran: Esfahan	GQ131334
8	*I. cappadocica* subsp. *steveniana*	Iran: Kordistan	GQ131335
9	*I. emarginata*	Iran: Khorasan	GQ131313
10	*I. gaubae*	Iran: Gorgan	GQ131314
11	*I. koeie*	Iran: Kohgiluyeh	GQ131315
12	*I. koelzii*	Iran: Khorasan	GQ131316
13	*I. kotschyana*	Iran: Tehran	GQ131317
14	*I. leuconeura*	Iran: Semnan	GQ131318
15	*I. lusitanica*	Iran: Markazi	GQ131319
16	*I. minima*	Iran: Kerman	GQ131320
17	*I. pachycarpa*	Iran: Kerman	GQ131321
18	*I. raphanifolia*	Iran: Tehran	GQ131322
19	*I. takhtajanii*	Iran: Kordistan	GQ131332
20	*I. indigotica*	China: Jiangsu	AF384104
21	*I. indigotica*	China: Xinjiang	AF384105
22	*I. tinctoria*	n. a.	DQ813301
23	*I. tinctoria*	n. a.	EF114671
24	*I. tinctoria*	n. a.	FJ593182
25	*I. trachycarpa*	Iran: Khorasan	GQ131324
26	*I. violascens*	China: Xinjiang	KJ623524
27	*I. violascens*	China: Xinjiang	KJ623525
28	*Aethionema arabicum*	n. a.	AY254539
29	*Aethionema elongatum*	n. a.	DQ518386
30	*Aethionema grandiflorum*	n. a.	DQ249867

of each primer, respectively, 0.5 μL DMSO, 1.5 mmol/L $MgCl_2$ and 2 U of r*Taq* polymerase (Takara Biotech Inc.). The ITS region was amplified using primers ITS5 and ITS4 designed by White *et al*.[31]. The amplification was performed under the following conditions: 5 min initial denaturation at 95 ℃, 35 cycles of 30 s denaturation at 95 ℃, 45 s annealing at 52 ℃, and 1 min elongation at 72 ℃, then a final elongation of 10 min at 72 ℃. PCR products were checked for length and concentrations on 1.2% agarose gel in TAE-buffer. Staining was done with non-toxic SYBR Green. Before sequencing, the PCR products were purified using a PCR product purification kit. Sequencing was performed on the automated ABI 3730 DNA Analyzer and the original amplification primers were used for double strand sequencing primers.

1.3 Phylogenetic analyses

The ITS sequences data was aligned using Clustal W as implemented in MEGA 5.0[32]. The best fitting nucleotide substitution model was selected by the Akaike information criterion (AIC) implemented in MrModeltest 2.3[33]. The K-2-p and gamma-distributed substitution rate heterogeneity was chosen as best fit model and applied to the ML and Bayesian analyses. Maximum parsimony tree was constructed using MEGA 5.0 under Tree-Bisection-Reconnection (TBR) searching method. Gaps were treated as missing data. Bootstrap values were calculated for 1000 replicates. Maximum Likelihood analysis was based on the K-2-p model. Neighbor-Join and BioNJ algorithms was used for heuristic search. A discrete Gamma distribution was used to model evolutionary rate differences among sites. Bootstrap values were calculated for 1 000 replicates. Bayesian phylogenetic tree was constructed using the BEAST software package V1.7.4[34]. Two independent runs of 5 million generations each were undertaken by sampling every 1000th generation. Each run was checked in Tracer v.1.5 for having reached a sufficient effective sample size (ESS over 100, as suggested by the authors) in relevant statistics. The two runs (log- and trees-files respectively) were then combined with LogCombiner v.1.7.4. Maximum clade credibility (MCC) tree was produced from the 90% post-burn-in trees using TreeAnnotator v.1.7.4. Finally, FigTree v1.5 was used to display and print molecular phylogenetic trees.

2 Results

2.1 ITS sequences analyses of *I. violascens*

The sequenced *I. violascens* ITS was 682 bp long. Nucleotide composition of the two ITS were listed in Table 2.

Table 2 Nucleotide composition of *I. violascens* ITS sequences

	T/%	C/%	A/%	G/%	Total/bp
I. violascens(26)	22.4	27.3	23.9	26.4	682
I. violascens(27)	22.4	27.3	23.9	26.4	682
Avg.	22.4	27.3	23.9	26.4	682

2.2 Sequences divergence of *Isatis*

The sequences of *I. violascens* were mostly identical. Sequences distance between *I. violascens* and other *Isatis* species were calculated with MGEA 5.0 using the K-2-p model and gamma distribution parameter (shape parameter = 1). The minimal distance was 0.2% found between *I. violascens* and *I. emarginata*, *I. minima*. The maximal distance was 7.3% found between *I. violascens* and *I. takhtajanii*. The mean distance within *Isatis* was 3.2%.

2.3 Phylogenetic analyses

The ITS alignment comprised 30 sequences and 629 characters, of which 438 were constant. Of the 188 variable characters, 146 were parsimony informative. The maximum parsimony analysis of ITS data matrix resulted in four equally most parsimonious (MP) trees with tree length of 265 (the consistency index=0.779, the retention index=0.892). The maximum likelihood analysis resulted in a maximum likelihood (ML) tree with log likelihood of −2 185.03. The topology of MP tree and ML tree differed in the phylogenetic position of group A and group B (Fig. 1). The trees obtained from Bayes method shared identical topology with that from ML method (Fig. 2). The strict consensus tree with bootstrap (BP) values and post-possibility (PP) values was shown in Fig. 2. As shown in the strict consensus tree, two clades were well-supported. Clade 1 included *I. emarginata*, *I. minima*, *I. trachycarpa*, *I. brevipes* and *I. violascens* with BP support of 56 and 63 for MP and ML analyses, respectively, PP support of 1.00. A strong supported subclade (subclade 3)

comprised of *I. emarginata*, *I. minima* and *I. violascens* was nest in clade 1. The support values for subclade was 99, 99 and 1.00. Clade 2 included the other species: *I. buschiana*, *I. gaubae*, *I. cappadocica*, *I. koelzii*, *I. indigotica*, *I. kotschyana*, *I. leuconeura*, *I. pachycarpa*, *I. takhtajanii*, *I. tinctoria*, *I. raphanifolia*, *I. lusitanica* and *I. koeie*. The clade 2 received BP and PP support of 68, 81 and 0.94.

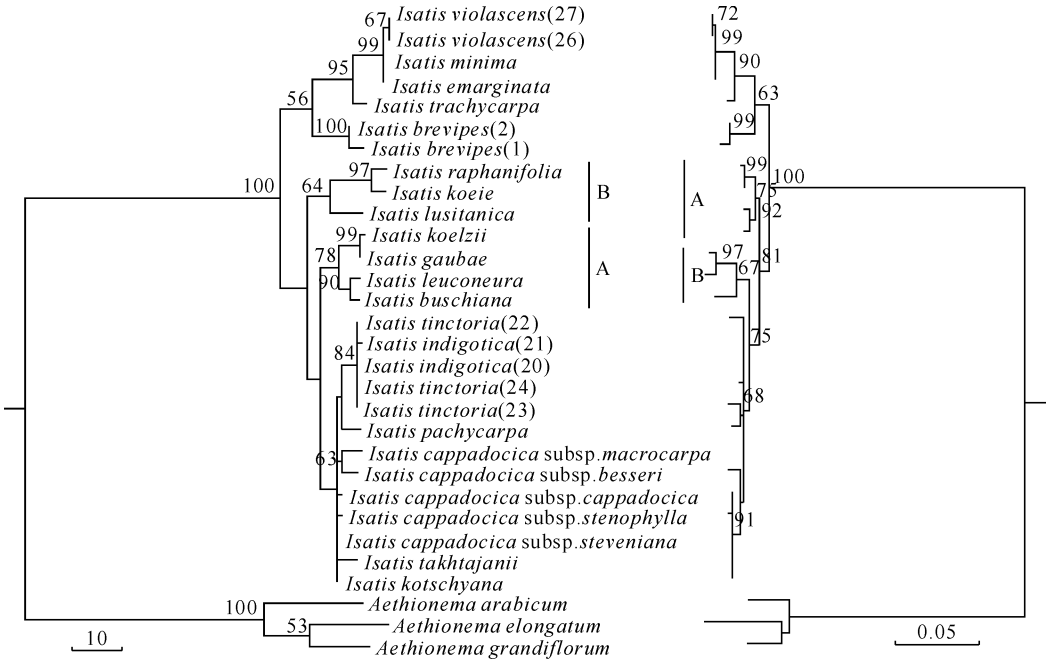

Fig. 1　Topology comparison between MP tree(left) and ML tree(right)

Bootstrap values above 50% are shown above the branches; The difference of MP and ML tree are marked by A and B

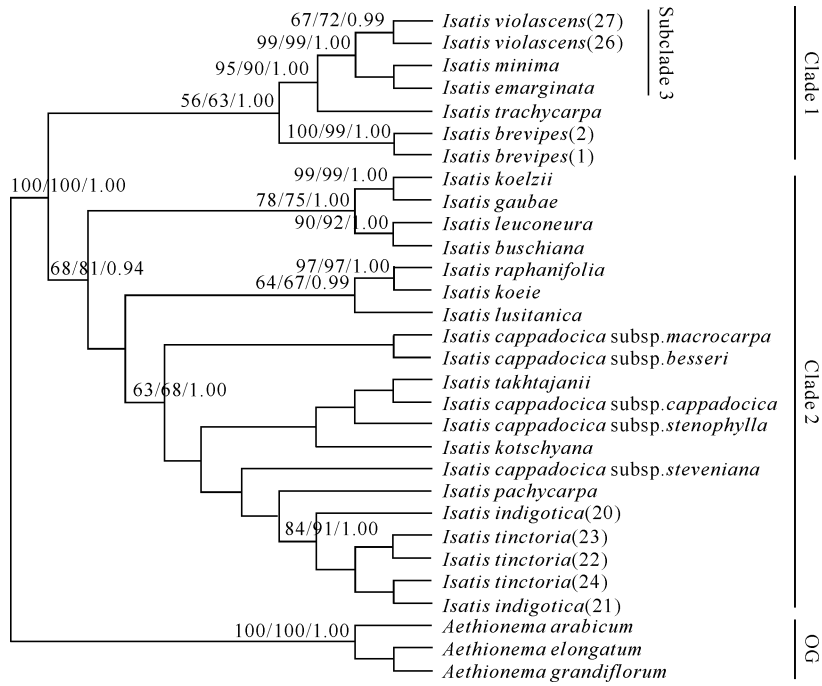

Fig. 2　The strict consensus phylogenetic tree constructed from ITS data based on Bayesian method

Bootstrap value (BP) and post-possibility value (PP) are above the branches; The first mentioned is BP value from MP method, the second mentioned is BP value from ML method, and the last mentioned is PP value.

Two clades, clade 1 and clade 2, are indicated, OG is abbreviation of outgroup

Table 3 Fruit character comparision of four *Isatis* species

Specie	Fruit	Shape	Size (mm, long×broad)	Hair/trichome	Wing
I. violascens	silicles	oblong-pandurate	(7～)8～10(～12)×(3.5～)4～5	densely puberulent with minute, simple, subclavate trichomes	subequally winged at base and margin. wings 1～2 mm wide at base and margin, 2～3 mm wide at apex.
I. emarginata	silicles	lamp-shaped	c. 9×c. 4.5	densely with minute simple hairs	narrowly equally winged all around with slightly thickened margins
I. minima	silicles	spatulate or oblanceolate	8～18×1～2	glabrous or pubescent with crisped trichomes	base and middle not winged, apex distinctly winged, wings 3～5 mm wide

2.4 Genetic distance within and between clades

The K-2-p genetic distance within clade 1, clade 2, and between them were calculated in MEGA 5.0. The mean distance within clade 1 and clade 2 was 2.2% and 2.0%, respectively. The distance within subclade 3 (among *I. emarginata*, *I. minima* and *I. violascens*) clade was 0.1%. Mean distance between the clade 1 and clade 2 was 5.0%. The overall mean distance within *Isatis* was 3.2%.

3 Discussion

3.1 Close relationship of *I. violascens* with *I. emarginata* and *I. minima*

Moazzeni[6] demonstrated that *Isatis* sensu Schulz was not monophyletic because genera *Pachypterygium*, *Tauscheria*, *Sameraria* and *Boreava* were nested in *Isatis* clade. As this paper was focused on the phylogenetic relationship of *I. violascens* within *Isatis*, thus genera *Pachypterygium*, *Tauscheria*, *Sameraria* and *Boreava* were not included. According to the present ITS phylogeny, *Isatis* was split into two well supported clades(Fig. 2). This result was consistent with studies of Moazzeni[6]. *I. violascens*, *I. emarginata* and *I. minima* formed a strong support subclade(subclade 3), with BP and PP value of 99, 99 and 1.00, respectively. The distance between *I. violascens* and *I. emarginata*, between *I. violascens* and *I. minima* was 0.2% each. The mean K-2-p distance within subclade 3 was very low, only 0.1%. The phylogeny result and low sequence divergence demonstrated a close relationship of *I. violascens* with *I. emarginata* and *I. minima*.

3.2 Taxonomic status of *I. violascens*

I. violascens and *I. emarginata* both have silicles fruit of similar size and densely with minute, simple hairs. The two species are differed slightly in the fruit shape and wings. The fruit of *I. violascens* are lamp-shaped and wings are subequally winged at base and margin. Otherwise, *I. emarginata* fruits are lamp-shaped and the wings are equally all around (Table 3). Based on fruits similarities, *I. violascens* was reduced to the synonym of *I. emarginata*(Flora of Pakistan).

Based on previous studies, species discrimination is considered to be successful if the minimum uncorrected interspecific p-distance involving a species was larger than its maximum intraspecific distance[35] or if all individuals of a species formed a monophyletic group in a phylogenetic tree[36]. Our data show that the intraspecific K-2-p distance of *I. violascens* was 0, smaller than the 0.2% interspecific distance between *I. violascens* and *I. emarginata*. Due to the limited samples of *I. violascens* and *I. emarginata*, we cannot demonstrate definitely the discrimination of *I. violascens* from *I. emarginata* because the sequence variation of *I. violascens* from other areas(eg. central Asia) cannot be excluded. Furthermore, the 0.2% interspecific distance between *I. violascens* and *I. emarginata* is much smaller than the mean distance(3.2%) within the genus. Therefore, from the evidence of similarities of morphology and low ITS sequences distance, we much more agree with the merge of the two species into one species and the reduction *I. violascens* to synonym of *I. emarginata*.

References:

[1] WARWICK S I, FRANCIS A, AL-SHEHBAZ I A. Brassicaceae: Species checklist and database on CD-Rom[J]. *Plant Systematics and Evolution*, 2006, 259: 249－258.

[2] APPEL O, AL-SHEHBAZ I A. Cruciferae[M]//KUBITZKI K. Families and Genera of Vascular Plants. Berlin-Heidelberg: Springer, 2003, 5: 75－174.
[3] DAVIS P H. *Isatis*[M]//DAVIS P H. Flora of Turkey and the East Aegean Islands. Edinburgh: Edinburgh University Press, 1965, 1: 287－307.
[4] AL-SHEHBAZ I A, BEILSTEIN M A, KELLOGG E A. Systematics and phylogeny of the Brassicaceae (Cruciferae): an overview[J]. *Plant Systematics and Evolution*, 2006, 259: 89－120.
[5] GERMAN D A, FRIESEN N, NEUFFER B, *et al*. Contribution to ITS phylogeny of the Brassicaceae, with special reference to some Asian taxa[J]. *Plant Systematics and Evolution*, 2009, 283: 33－56.
[6] MOZAAENI H, ZARRE S, AL-SHEHBAZ I A, *et al*. Phylogeny of *Isatis* (Brassicaceae) and allied genera based on ITS sequences of nuclear ribosomal DNA and morphological characters[J]. *Flora*, 2010, 205: 337－343.
[7] WARWICK S I, MUMMENHOFF K, SAUDER C A, *et al*. Closing the gaps: phylogenetic relationships in the Brassicaceae based on DNA sequence data of nuclear ribosomal ITS region[J]. *Plant Systematics and Evolution*, 2010, 285: 209－232.
[8] DVORAK F. On the evolutionary relationship in the family Brassicaceae[J]. *Feddes Repertorium*, 1971, 82: 357－372.
[9] HEDGE I. A systematic and geographical survey of the Old World Cruciferae[M]//VAUGHAN J G, MACLEOD A J. The biology and chemistry of the Cruciferae. New York: Academic Press, 1976.
[10] KOCH M, AL-SHEHBAZ I A, MUMMENHOFF K. Molecular systematics, evolution, and population biology in the mustard family (Brassicaceae)[J]. *Annals of the Missouri Botanical Garden*, 2003, 90: 151－171.
[11] MEYER F K. Conspectus der "*Thlaspi*"-Arten Europas, Afrikas und Vorderasiens[J]. *Feddes Repertorium*, 1973, 84: 449－469.
[12] KOCH M, BISHOP J, MITCHELL T. Molecular systematics and evolution of *Arabidopsis* and *Arabis*[J]. *Plant Biology*, 1999, 1: 529－537.
[13] MEYER F. Seed-coat anatomy as a character for a new classification of *Thlaspi*, Contributiones selectae ad floram et vegetationem orientis[C]. Proceedings of the Third Plant Life of Southwest Asia Symposium, 1990: 3－8.
[14] HEDGE I C. *Lepidieae*[M]//RECHINGER K H. Flora Iranica. Graz: Akademische Druck－und Verlagsanstalt, 1968, 57: 63－122.
[15] JAFRI S M H. Brassicaceae[M]//NASIR E, ALI S L. Flora of West Pakistan. Karachi: University of Karachi, 1973, 55: 1－308.
[16] HEBERT P D N, CYWINSKA A, BALL S L, *et al*. Biological identifications through DNA barcodes[J]. *Proceedings of the Royal Society B-Biological Sciences*, 2003, 270: 313－321.
[17] SAVOLAINEN V, COWAN R S, VOGLER A P, *et al*. Towards writing the encyclopaedia of life: an introduction to DNA barcoding[J]. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, 2005, 360: 1 805－1 811.
[18] DEDALL R. Species discovery versus species identification in DNA barcoding efforts: Response to Rubinoff[J]. *Conservation Biology*, 2006, 20: 1 545－1 547.
[19] HAJIBABAEI M, SINGER G A C, HEBERT P D N, *et al*. DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics[J]. *Trends in Genetics*, 2007, 23: 167－172.
[20] SMITH M A, FISHER B L, HEBERT P D N. DNA barcoding for effective biodiversity assessment of a hyperdiverse arthropod group: the ants of Madagascar[J]. *Philosophical Transactions of the Royal Society B-Biological Sciences*, 2005, 360: 1 825－1 834.
[21] WILL K W, MISHLER B D, WHEELER Q D. The perils of DNA barcoding and the need for integrative taxonomy[J]. *Systematic Biology*, 2005, 54: 844－851.
[22] HOLLINGSWORTH P M, GRAHAM S W, LITTLE D P. Choosing and Using a Plant DNA Barcode[J]. *PLoS One*, 2011, e6.
[23] GERMAN D A, GRANT J R, LYSAK M A, *et al*. Molecular phylogeny and systematics of the tribe *Chorisporeae* (Brassicaceae)[J]. *Plant Systematics and Evolution*, 2011, 294: 65－86.
[24] JAEN-MOLINA R, CAUJAPE-CASTELLS J, *et al*. The molecular phylogeny of *Matthiola* R. Br. (Brassicaceae) inferred from ITS sequences, with special emphasis on the Macaronesian endemics[J]. *Molecular Phylogenetics and Evolution*, 2009, 53: 972－981.
[25] JORDON-THADEN I, HASE I, AL-SHEHBAZ I, *et al*. Molecular phylogeny and systematics of the genus *Draba* (Brassicaceae) and identification of its most closely related genera[J]. *Molecular Phylogenetics and Evolution*, 2010, 55: 524－540.
[26] WARWICK S I, SAUDER C A, AL-SHEHBAZ I A. Phylogenetic relationships in the tribe *Alysseae* (Brassicaceae) based on nuclear ribosomal ITS DNA sequences[J]. *Botany*, 2008, 86: 315－336.
[27] COUVREUR T L P, FRANZKE A, AL-SHEHBAZ I A, *et al*. Molecular phylogenetics, temporal diversification, and principles of evolution in the mustard family (Brassicaceae)[J]. *Molecular Biology and Evolution*, 2010, 27: 55－71.
[28] FRANZKE A, LYSAK M A, AL-SHEHBAZ I A, *et al*. Cabbage family affairs: the evolutionary history of Brassicaceae[J]. *Trends in Plant Science*, 2011, 16: 108－116.
[29] MATHEWS S, MCBREEN K. Phylogenetic relationships of B-related phytochromes in the Brassicaceae: Redundancy and the persistence of phytochrome D[J]. *Molecular Phylogenetics and Evolution*, 2008, 49: 411－423.
[30] DOYLE J J. A rapid DNA isolation procedure for small quantities of fresh leaf tissue[J]. *Phytochemical Bulletin*, 1987, 19: 11－15.
[31] WHITE T J, BRUNS T, LEE S, *et al*. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics[M]//INNIS M A, GELFAND D H, SNINSKY J J. PCR protocols: A guide to methods and applications. New York: Academic Press, 1990: 315－322.
[32] TAMURA K, PETERSON D, PETERSON N, *et al*. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods[J]. *Molecular Biology and Evolution*, 2011, 28: 2 731－2 739.
[33] NYLANDER J. MrModeltest v2: Program distributed by the author[M]. Evolutionary Biology Centre, Uppsala University, 2004.
[34] DRUMMOND A J, SUCHARD M A, XIE D, *et al*. Bayesian phylogenetics with BEAUti and the BEAST 1. 7[J]. *Molecular Biology and Evolution*, 2012: 1－5.
[35] CBOL Plant Working Group. A DNA barcode for land plants[J]. *Proceedings of the National Academy of Sciences of the United States of America*, 2009, 106: 12 794－12 797.
[36] HOLLINGSWORTH M L, CLARK A A, FORREST L L, *et al*. Selecting barcoding loci for plants: evaluation of seven candidate loci with species-level sampling in three divergent groups of land plants[J]. *Molecular Ecology Resources*, 2009, 9: 439－457.