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ORIGINAL ARTICLE

Commercialized non-*Camellia* tea: traditional function and molecular identification

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Abstract Non-*Camellia* tea is a part of the colorful Chinese tea culture, and is also widely used as beverage and medicine in folk for disease prevention and treatment. In this study, 37 samples were collected, including 33 kinds of non-*Camellia* teas and 4 kinds of teas (*Camellia*). Traditional functions of non-*Camellia* teas were investigated. Furthermore, non-*Camellia* teas of original plants were characterized and identified by molecular methods. Four candidate regions (*rbc*L, *mat*K, ITS2, *psb*A-*trn*H) were amplified by polymerase chain reaction. In addition, DNA barcodes were used for the first time to discriminate the commercial non-*Camellia* tea and their adulterants, and to evaluate their safety. This study showed that BLASTN and the relevant phylogenetic tree are efficient tools for identification of the commercial non-*Camellia* tea and their adulterants. However, some sequences from original plants have not been found and there is a limitation of sequence number of original plants in GenBank. Submitting more original plant sequences to the GenBank will be helpful for evaluating the safety of non-*Camellia* teas.

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1. Introduction

Tea (the leaves from *Camellia* plants) has been one of the most widely consumed non-alcoholic beverages in the world for thousands of years. It plays important roles in commerce, health, and culture. However, many other kinds of plants have been widely used as tea as well. These are not from *Camellia* (Theaceae), and are called non-*Camellia* tea, such as kuding tea, huangqin tea, laoying tea[1,2]. More than 20 kinds of non-*Camellia* tea are reportedly used within the Chinese culture[3]. Modern pharmacology studies have reported that non-*Camellia* tea may prevent and/or treat chronic metabolic diseases, by producing hypolipidemic, hypoglycemic, or hypotensive actions. Moreover, most kinds of non-*Camellia* tea have also been used as medicine for disease prevention and treatment in folklore[4–6].

In recent years, with the increasing international demand for herbal medicines, non-*Camellia* tea has attracted increasing attention. However, original plants of non-*Camellia* tea are confused, and some adulterants have begun to appear in the market. Furthermore, fatalities and serious illnesses have occurred after drinking non-*Camellia* teas, caused by overdose, mislabeled products, or allergic reactions[7,8]. For instance, kuding tea is suitable for high blood pressure, body fat or hot body, but not for the person whose body 'slants cold' in traditional Chinese medicine theory. According to this theory, a person whose body 'slants cold' will receive no improvement from ingestion of kuding tea and symptoms may appear or worsen, including abdominal pain, severe diarrhea and other symptoms[9]. The significant differences in chemical components among different kinds of the original plants could account for such variations in responses. Finally, undefined compounds in some of these teas may be dangerous to health.

Identification of non-*Camellia* tea is difficult, partly due to a lack of unified standards. Traditionally, morphological features remain as the main basis of taxonomy[10]. However, many of these commercial products are dried and processed, rendering the authentication by morphological methods very difficult. When morphological characteristics are absent, a DNA barcoding technique can identify and detect species utilizing one or a few DNA fragments[11]. DNA barcoding technique is a supplement to traditional authentication method which has been able to solve some identification problems[11–13]. In this study, we randomly collected non-*Camellia* tea from the medicinal material market in China. Using DNA barcoding technique, the original plants from which the teas were derived were identified to (1) explore which DNA regions are better for the authentication of non-*Camellia* tea traditionally used by Chinese people and (2) evaluate their safety.

2. Materials and methods

2.1. Plant materials

We collected 37 commercialized tea samples, including 33 kinds of non-*Camellia* tea and 4 kinds of *Camellia* tea from 16 provinces (Yunnan, Sichuan, Guangxi, etc.) in China during 2012, and recorded the detailed information of these medicinal non-*Camellia* tea samples, including the local tea name, collecting location and time, and therapeutic effects (Table 1). All the samples were pressed and deposited at the Herbarium of the Institute of Medicinal Plant Development (IMPLAD).

2.2. DNA barcoding

Four candidate barcodes (*rbc*L, *mat*K, *psb*A-*trn*H and ITS2) were selected based on previous barcoding studies[71–73]. We isolated the total genomic DNA from approximately 100 mg of dried powder from each sample using the cetyl trimethylammonium bromide method.[74] Extracted DNA was stored in sterile microcentrifuge tubes at $-20\ ^{\circ}C$.

The selected regions were amplified by polymerase chain reaction (PCR) on a PCR system 9700 thermocycler (Gene Co., USA). DNA was amplified in 20 μL of reaction mixtures containing 1 U ExTaq polymerase with 10 × ExTaq buffer (100 mmol/L pH 8.3 Tris–HCl, 500 mmol/L of KCl) (Takara, China), 1.25 mmol/L of deoxyribonucleotide triphosphate, 0.05 mmol/L of each primer, and 20 ng of template DNA. Primers and reaction conditions used in the present study were listed in Table 2. The amplified products were sequenced in forward directions with the primers used for amplification in the Beijing Genomics Institute (China). Sequences were assembled and aligned using Bioedit Sequence Alignment editor version 7.0.9.

2.3. BLASTN and phylogenetic analysis

BLASTN and the nearest distance method were used to identify obtained relative accurate identification of species. First, the measured DNA sequences from non-*Camellia* tea were determined using BLASTN[75] against the NCBI databases to identify the original plants of non-*Camellia* tea with similarity over 95%. To optimize correct identifications, DNA sequences of four candidate regions (*rbc*L, *mat*K, ITS2, *psb*A-*trn*H) from non-*Camellia* tea were determined from the best reciprocal hits. In most cases this corresponded to the sequence with the highest BLAST score. Second, in order to find a suitable reference sequence, all of *rbc*L, *mat*K, *psb*A-*trn*H, and ITS2 were extracted from the National Center for Biotechnology Information (NCBI) database according to the names of origin plant of the non-*Camellia* tea. After cluster and phylogenetic analysis, individual sequences were eliminated because of their ambiguous nucleotides shorter than 100 bp. Finally, the download sequences including 29 *rbc*L, 26 *mat*K. 22 *psb*A-*trn*H, and 29 ITS2 (Table 3) combining with the sequences of commercial non-*Camellia* tea were used to construct phylogenetic trees by Mega 5.0[76] and Clustal X[77] with a bootstrap value of 1000 replicates, respectively. Preliminary trees were reconciled by setting the bootstrap value greater than 50%, yielding a more credible consensus tree.

3. Results

3.1. Traditional uses

According to the literature (Table 1), original plants of 33 kinds of non-*Camellia* tea are distributed across 29 genera in 22 families. The most widely used plant portions are leaves (26), followed by flowers (7), herbs (3), stems (3), and the least used plant portions are seeds (1) and branches (1). The investigated non-*Camellia* teas have a variety of therapeutic applications (Table 1). The non-*Camellia* teas have been mainly used for three therapeutic effects: (I) heat-clearing tea (20), such as vine tea, qingqiangliu tea, yeju tea; (II) digestant tea (8), such as laoying tea, zhegu tea, liangwang tea; (III) health tea (9), such as jiaogulan tea, kuqiao tea, lvluohua tea.

Table 1 Summary of sample collecting location and time, original plants and traditional function of non-*Camellia* tea.

Local commodity name	Collecting location and time	Original plant	Use part	Traditional function	Reference
Non-*Camellia* tea					
Baixue tea	Yunnan Province. May 2012	*Thamnolia vermicularis* (Ach.) Asahina *T. subuliforms* (Ehrh.) W. Culb.	Leaves	Clearing away heat and remove toxic material, relieving cough, reducing sputum, anti-inflammatory.	14,15
Big leaf kuding old tea	Guangxi Province June 2012	*Ilex latifolia* Thunb. *I. kaushue* S. Y. Hu	Leaves	Quenching thirst, improving eyesight, relieving restlessness, refreshing oneself, dissolving phlegm, increasing secretion of urine, relieving sore throat.	6,16,17
Big leaf kuding tender tea	Guangxi Province June 2012	*Ilex latifolia* Thunb. *I. kaushue* S. Y. Hu	Leaves	Quenching thirst, improving eyesight, relieving restlessness, refreshing oneself, dissolving phlegm, increasing secretion of urine, relieving sore throat.	6,16,17
Duosuike sweet tea	Sichuan Province May 2012	*Lithocarpus litseifolius* (Hance) Chun	Leaves	Reducing fever and causing diuresis, nourishing the liver and kidney, regulating the stomach to descend stomach-qi, moistening the lung to arrest cough.	18–20
Fengwei tea	Wenshan, Yunnan Province May 2012	*Elsholtzia bodinieri* Vant. *E. heterophylla* Diels	Leaves	Relieving exterior syndrome by dispersion, regulating flow of qi, harmonizing the stomach.	21
Gongju tea	Huangshan city, An'hui Province July 2012	*Chrysanthemum morifolium* Ramat	Flowers	Expelling wind and clearing away heat, clearing liver-fire to treat eye disease, and eliminating toxic substances.	22
Guangxi sweet tea	Guangxi Province June 2012	*Rubus suavissimus* S. Lee	Leaves, and branch	Clearing away heat and removing toxic material, promoting the secretion of saliva or body fluid, moistening the lung, relieving a cough, relieving sore throat.	23–25
Hongxue tea	Yunnan Province May 2012	*Lethariella cashmeriana* Korw. *L. cladonioides* (Nyl.) Krog; *L. sernanderi* (Motyka) Obermayer; *L. zahlbruckneria* (Dr.) Krog.	Leaves	Clearing heart-fire to regain consciousness, relieving pain, hyperlipidemia, anti-fatigue, anti-inflammatory.	14,15,26,27
Huangqin tea	Inner Mongolia Autonomous Region September 2012	*Scutellaria baicalensis* Georgi; *S. scordifolia* Fisch. ex Schrank; *S. amoena*. C. H. Wright; *S. viscidula* Bunge	Herbs	Heat-clearing and damp-drying drug, purging fire for removing toxin, anti-inflammatory, promoting digestion.	3,28
Jiaogulan tea	Hunan Province July 2012	*Gynostemma pentaphyllum* (Thunb.) Makino	Leaves	Anti-fatigue, anti-hypoxia, enhancing immu-neity, hyperglycemic and hypolipidemic.	29–31
Kuqiao tea	Liangzhou, Sichuan Province May 2012	*Fagopyrum tataricum* (L.) Gaertn.	Seeds	Hyperglycemic, hypolipidemic, enhancing immunity, et al.	32
Laoying tea	Guangyuan, Sichuan Province May 2012	*Litsea coreana* Levl. Var. lanuginose *Actinodaphne cupularis* (Hemsl.) Gemble	Leaves	Diabetes, expelling dampness, anti-diarrhea, stop burping, promoting digestion, et al.	33–36

Table 1 (*continued*)

Local commodity name	Collecting location and time	Original plant	Use part	Traditional function	Reference
Liangwang tea	Yunnan Province May 2012	*Nothopanax delavayi* (Franch.) Harms ex Diels	Leaves, and flowers	Clearing away heat and removing toxic material, relaxing muscles and bones, promoting digestion, et al.	37
Luobuhongma tea	Xinjiang Uygur Autonomous Region July 2012	*Apocynum venetum* L.	Leaves, and flower	Hypotensive, anti-radiation, anti-aging, preventing bronchitis and cold.	38–40
Luobubaima tea	Xinjiang Uygur Autonomous Region July 2012	*Apocynum hendersonii* Hook.	Leaves, and flowers	Reducing fever and causing diuresis, flat liver resting to restore energy, hypotensive, hypolipidemic, anti-inflammatory, anti-anaphylaxis.	39,41
Luohan tea	Guangxi Province June 2012	*Engelhardtia roxburghiana*. Wall.	Leaves	Clearing away heat and removing toxic material, engendering liquid and allaying thirst, relieving summer-heat, removing dampness.	42
Lvluohua tea	Tibet Autonomous Region July 2012	*Epipremnum aureum* (Linden & André) G. S. Bunting	Flowers	Hypoglycemic, anti-bacterial, anti-inflammatory, hypotensive.	43,44
Mabiancao tea	Shanxi Province June 2012	*Verbena officinalis* L.	Herbs	Clearing away heat and removing toxic material, promoting blood circulation to induce menstrual, diuretic swelling, preventing attack of malaria.	45
Niubaiteng sweet tea	Guangxi Province June 2012	*Hedyotis hedyotidea* (DC.) Merr.	Stems, leaves	Clearing away heat, dispelling wind, eliminating dampness, detumescence detoxification, et al.	46,47
Paraguay tea	Bei Jing August 2012	*Ilex paraguariensis* St. Hilaire.	Leaves	Curing dyspepsia, antiobesity effect.	48–50
Qingqianliu tea	Jiangxi Province August 2012	*Cyclocarya paliurus* (Batal.) Iljinsk.	Leaves	Engendering liquid and allaying thirst, clearing away heat and removing toxic material, enhancing physical strength, prolonging life.	51–53
Sishi tea	Wuyuan, Jiangxi Province August 2012	*Scoparia dulcis* L.	Herbs	Dispelling wind and relieving cough, clearing away heat, removing dampness by dieresis.	54
Shen tea	Yunnan Province May 2012	*Clerodendranthus spicatus* (Thunb.) C. Y. Wu ex H. W. Li	Leaves	Clearing heat and expelling damp, removal of stone and increasing secretion of urine.	55
Shiliang tea	Guangxi Province June 2012	*Chimonanthus salicifolius* S. Y. H; *C. Zhejiangensis* M. C. Liu; *C. nitens* Oliv.	Leaves	Dispelling wind to relieve exogenous syndrome, regulating qi-flowing for strengthening spleen, anti-diarrhea.	56,57
Shiya tea	Guangxi Province June 2012	*Adinandra nitida* Merr. ex Li	Leaves	Engendering liquid and allaying thirst, anti-inflammatory, clearing away heat and removing toxic material.	58–60
Small leaf kuding tea	Sichuan Province. May 2012	*Ligustrum robustum* (Roxb.) Blume	Leaves	Cooling and refreshing antipyretic, dieresis.	6,17,61
Tianyeju tea	Yunnan Province May 2012	*Stevia rebaudiana* Bertoni	Leaves	Helping to produce saliva and slake thirst, hypotensive, hypoglycemic.	62
Vine tea	Zhangjiajie, Hunan Province June 2012	*Ampelopsis grossedentata* (Hand.-Mazz) W. T. Wang	Stems, leaves	Clearing away heat and removing toxic material, diminishing inflammation and relieving sore throat, hypotensive and hypolipidemic.	63,64
Xiangsiteng tea	Guangxi Province June 2012	*Abrus precatorius* Linn.	Stems, leaves	Helping to produce saliva, moistening lung, clearing heat, induce diuresis diuresis.	65,66
Xiangfeng tea	Hebei Province July 2012	*Chimonanthus salicifolius* S. Y. Hu	Leaves	Promoting digestion, treating liver-stomach disharmony, et al.	57

Table 1 (*continued*)

Local commodity name	Collecting location and time	Original plant	Use part	Traditional function	Reference
Yaowang tea	Xi'an, Shangxi Province July 2012	*Potentilla fruticosa* L. *P. glabra* Lodd. var. mandshurica	Leaves, flowers	Clearing away heat, invigorating the stomach, regulating the menstrual function.	67
Yeju tea	Zhejiang Province August 2012	*Chrysanthemum indicum* L.	Flowers	Clearing away heat and removing toxic material, dispersing wind and heat, dispersing blood stasis, improving eyesight, hypotensive, et al.	62
Zhegu tea	Wanning, Hainan Province. August 2012	*Mallotus oblongifolius* (Miq.) Muell. Arg	Leaves	Neutralizing the greasy, promoting digestion, eliminating summer-heat, prevention and treatment of common cold.	68
Traditional tea					
Black tea	Fujian Province March 2012	*Camellia sinensis*	Leaves	Strengthening tendons and bones, anti-fatigue, preventing cold.	69
Green tea	Chongqing Province June 2012	*Camellia sinensis*	Leaves	Refreshing oneself, resolving phlegm, promoting digestion, inducing diuresis, detoxify.	6
Tieguanyin tea	Fujian Province March 2012	*Camellia sinensis*	Leaves	Exciting the brain, inducing diuresis strong heart, anti-aging, anticancer and detoxification.	70
Xihulongjing tea	Hangzhou, Zhejiang Province August 2012	*Camellia sinensis*	Leaves	Refreshing oneself, resolving phlegm, promoting digestion, inducing diuresis, detoxify.	6

Table 2 Primers and reaction conditions used in the present study.

Gene name	Name of primer and primer sequence 5′-3′	PCR reaction condition
*rbc*L	724R: TCGCATGTACCTGCAGTAGC 1F: ATGTCACCACAAACAGAAAC	95 °C 5 min 94 °C 30 s, 56 °C 30 s, 72 °C 100 s, 35 cycles 72 °C 7 min
*mat*K	3F: CGTACAGTACTTTTGTGTTTACGAG 1R: ACCCAGTCCATCTGGAAATCTTGGTTC	95 °C 5 min 95 °C 30 s, 52 °C 30 s, 72 °C 100 s, 32 cycles 72 °C 7 min
*psb*A-*trn*H	*trn*H: CGCGCATGGTGGATTCACAATCC *psb*A: GTTATGCATGAACGTAATGCTC	94 °C 4 min 94 °C 30 s, 58 °C 45 s, 72 °C 100 s, 32 cycles 72 °C 7 min
ITS2	ITS2F: ATGCGATACTTGGTGTGAAT ITS2R: GACGCTTCTCCAGACTACAAT	95 °C 5 min 95 °C 30 s, 56 °C 30 s, 72 °C 100 s, 35 cycles 72 °C 7 min

3.2. *DNA extraction, PCR, and sequencing success*

All samples were extracted through CTAB method successfully. At the same time, the PCR success rates of *rbc*L, *mat*K, *psb*A-*trn*H and ITS2 were 91.9% (34/37), 75.8% (28/37), 78.4% (29/37) and 100% (37/37), respectively. All the PCR products were sequenced successfully. In all sequences, *rbc*L sequence lengths ranged from 625 bp to 692 bp; *mat*K sequence lengths ranged from 780 bp to 860 bp. *psb*A-*trn*H sequence lengths were from196 bp to 587 bp, and ITS2 sequence lengths were from 426 bp to 506 bp.

3.3. *Species identification based on BLASTN*

The *rbc*L, *mat*K, *psb*A-*trn*H, and ITS2 sequences of the non-*Camellia* teas were also blasted against the NCBI database with maximum identity that are greater than 95% using an *e*-value below 0.0 to determine the difference between the original plants. The closest match in the database was recorded and DNA sequences of non-*Camellia* tea were determined from the reciprocal best hits.

Taking into account the uncertainties arising from incomplete databases, shared barcodes, ambiguous common names and

Table 3 The sequence information in GenBank.

Species	Genbank no.			
	*rbc*L	*mat*K	*psb*A-*trn*H	ITS2
Abrus precatorius (1–2)	JN407285; JF738654	JN407125	JN406972	AF467015
Actinodaphne cupularis	–	–	–	HQ697213
Apocynum venetum	–	–	–	DQ449485
Ampelopsis grossedentata	JQ182479	JF953244;	JF437070	–
Camellia sinensis (1–2)	JN654337	AJ429305; JN654321	GQ487359	FJ004887
Chimonanthus nitens	–	–	–	AY786094
Chimonanthus Zhejiangensis	–	AY525341	–	AY786106
Chimonanthus salicifolius	HQ427177	HQ427325	HQ427018	AY786102
Chrysanthemum x morifolium	–	EU334382; HM989758	EF091621	EF091597
Chrysanthemum indicum	JF949971	–	JF949971	EF577298; JN315940
Clerodendranthus spicatus	GQ464985	FJ513161	FJ513103; GQ464982	HM595465
Cyclocarya paliurus (1–2)	AY263942; AY147094	AY147098	–	AF179583
Dasiphora fruticosa	–	AB458578	JN044379	–
Engelhardia roxburghiana	–	–	–	AF303801
Epipremnum aureum (1–2)	JQ734504; JN090003	JN090088	–	–
Fagopyrum tataricum	JN187117	JF829984	JQ807577	AB000339
Gynostemma pentaphyllum	AY968523	AY968451	EF621687	FJ980303
Hedyotis hedyotidea	HM752999	HM753079	HM640314; HM640334	HQ148756
Ilex aquifolium	–	JN896160	–	–
Ilex kaushue	JF942007	JF954101	JN044945	–
Ilex latifolia	JF942011	HQ427289	JN044949	AF200592; AY140215
Ilex paraguariensis	FJ394634	GQ248141	GQ248322	FJ394705
Lethariella cashmeriana	–	–	–	AF297743; DQ980014
Lethariella sernanderi	–	–	–	AF297744
Ligustrum robustum	JF942292	JF954385	JN045240	–
Litsea coreana	–	–	–	AF272286
Lithocarpus litseifolius	–	EF057121	–	EF057112
Mallotus oblongifolius	JF738963	–	–	–
Poacynum pictum	–	–	–	DQ451830
Potentilla fruticosa	PFU06818	–	AM114863	AF163478
Scutellaria amoena	HQ676585	JX981408	HQ680371	–
Scutellaria baicalensis	GQ374130	JX981417; HQ676586; FJ513169	HQ680366	JF421544; FJ609732
Scutellaria scordifolia	HM590110	HQ839713	FJ513143	FJ546875
Scutellaria viscidula	HQ676583	HQ676587	HQ680369	–
Scoparia dulcis	JQ593281	JQ588687; JQ588683	–	AY963776
Stevia rebaudiana	AY215182	AY215865	AY215611	AB457301
Thamnolia vermicularis	–	–	–	JQ409350
Verbena officinalis (1–3)	HM850444; JF950020; JN893754	HM850974	GQ435188; HE966861	GQ434586

–Species without the gene sequence in NCBI.

sequencing without success, BLASTN analysis of the *rbc*L data showed 32 commercialized samples were assigned to species, one sample to genus, and one sample was not recorded in the GenBank (Table 3). But because *rbc*L identification ability is limited, only 11 commercialized samples matched with the original plants, including 7 kinds of non-*Camellia* teas and 4 kinds of traditional tea (Table 4).

The *mat*K analysis data showed 24 commercialized samples were assigned to species, 4 to family. As one common primer of DNA barcoding, *mat*K is suitable for genera identification. In this report, only 13 commercialized samples matched the designation of the original plants: 11 kinds of non-*Camellia* teas and 2 kinds of traditional teas (Table 4).

BLASTN analysis of the *psb*A-*trn*H data indicated 26 samples were assigned to species, 1 to family, and 2 were not recorded in GenBank. Only 13 commercialized samples matched the designation of the original plants: 12 kinds of non-*Camellia* teas and 1 kind of traditional tea (Table 4).

The ITS2 BLASTN result indicated 30 commercialized samples were assigned to species, 4 to genera, 3 to family. Of these, 23 commercialized samples matched the designation of the original

Table 4 BLASTN identification result of non-*Camellia* tea.

No.	Commodity tea name	*rbc*L	*mat*K	*psb*A-*trn*H	ITS2
BYC-1	Paraguay tea	*Ilex sp.*	*Ilex aquifolium*	*Ilex paraguariensis*#	*Ilex paraguariensis*#
BYC-2	Baixue tea	*Ampelopsis brevipedunculata*	*Ampelopsis grossedentata*	*Ampelopsis grossedentata*	Caprifoliaceae
BYC-3	Blank tea	*Camellia sinensis*#	*Camellia sinensis*#	*Camellia cuspidata*	*Camellia sinensis*#
BYC-4	Big leaf Kuding tender tea	*Ilex kaushue*#	*Ilex aquifolium*	*Ilex pentagona*	*Ilex latifolia*#
BYC-5	Big leaf Kuding old tea	N	N	N	*Ilex latifolia*#
BYC-6	Duosuike sweet tea	*Quercus nigra*	Fagaceae	*Quercus phillyraeoides*	*Lithocarpus sp.*
BYC-7	Fengwei tea	*Elsholtzia stauntonii*	*Mosla chinensis*	*Mosla chinensis*	Lamiaceae
BYC-8	Gongju tea	*Chrysanthemum mutellinum*#	*Chrysanthemum* × *morifolium*#	*Chrysanthemum indicum*	*Chrysanthemum morifolium*#
BYC-9	Green tea	*Camellia sinensis*#	Compositae	*Camellia cuspidata*	*Camellia sp.*
BYC-10	Guangxi sweet tea	N	N	N	*Rubus crataegifolius*
BYC-11	Hongxue tea	*Panax ginseng*	N	N	*Scutellaria baicalensis*
BYC-12	Huangqin tea	*Scutellaria rehderiana*	*Scutellaria baicalensis*#	*Scutellaria baicalensis*#	*Scutellaria baicalensis*#
BYC-13	Jiaogulan tea	*Gynostemma pentaphyllum*#	*Gynostemma pentaphyllum*#	*Gynostemma pentaphyllum*#	*Gynostemma pentaphyllum*#
BYC-14	Kuqiao tea	*Panax ginseng*	N	N	*Stevia rebaudiana*
BYC-15	Laoying tea	*Litsea japonica*	*Cinnamomum brenesi*	N	*Litsea coreana*#
BYC-16	Liangwang tea	*Macropanax dispermus*	*Schefflera heptaphylla*	*Metapanax delavayi*#	*Metapanax delavayi*#
BYC-17	Luobuhongma tea	*Apocynum cannabinum*	*Apocynum cannabinum*	*Apocynum cannabinum*	*Apocynum venetum*#
BYC-18	Luobubaima tea	*Apocynum cannabinum*	*Apocynum cannabinum*	*Apocynum cannabinum*	*Poacynum hendersonii*#
BYC-19	Luohan tea	*Engelhardtia fenzelii*	*Alfaropsis roxburghiana*#	Unknown	*Engelhardtia roxburghiana*#
BYC-20	Lvluohua tea	*Edgeworthia chrysantha*	Thymelaeaceae	Thymelaeaceae	*Edgeworthia chrysantha*
BYC-21	Mabiancao tea	*Verbena bracteata*	*Verbena rigida*	*Verbena stricta*	*Verbena officinalis*#
BYC-22	Niubaiteng sweet tea	*Hedythyrsus sp.*	*Hedyotis hedyotidea*#	*Hedyotis hedyotidea*#	*Hedyotis hedyotidea*#
BYC-23	Qingqianliu tea	Unknown	*Cyclocarya paliurus*#	Unknown	*Cylocarya paliurus*#
BYC-24	Sishi tea	*Bacopa sp.*	N	*Gratiola neglecta*	*Callicarpa poilanei*
BYC-25	Shen tea	*Clerodendranthus spicatus*#	N	N	*Orthosiphon aristatus*#
BYC-26	Shiliang tea	N	*Chimonanthus salicifolius* S. Y. H#	*Chimonanthus salicifolius*#	*Chimonanthus salicifolius* S.Y. H#
BYC-27	Shiya tea	*Panax ginseng*	N	N	*Adinandra elegans*
BYC-28	Tianyeju tea	*Stevia rebaudiana*#	*Stevia rebaudiana*#	*Stevia rebaudiana*#	*Stevia rebaudiana*#
BYC-29	Tieguanyin tea	*Camellia sinensis*#	Compositae	*Camellia chekiangoleosa*	*Camellia sp.*
BYC-30	Vine tea	*Ampelopsis brevipedunculata*	*Ampelopsis grossedentata*#	*Ampelopsis grossedentata*#	Compositae
BYC-31	Xihulongjing tea	*Camellia sinensis*#	*Camellia sinensis*#	*Camellia sinensis*#	*Camellia sinensis*#
BYC-32	Xiangsiteng tea	*Abrus precatorius*#	*Abrus precatorius*#	*Abrus precatorius*#	*Abrus precatorius*#
BYC-33	Xiangfeng tea	*Panax ginseng*	*Chimonanthus salicifolius*#	*Chimonanthus salicifolius*#	*Chimonanthus Zhejiangensis*#
BYC-34	Small leaf Kuding tea	*Panax ginseng*	N	*Ligustrum robustum*#	*Ligustrum robustum*#
BYC-35	Yaowang tea	*Potentilla fruticosa*#	*Draba lanceolata*	N	*Dasiphora phyllocalyx*
BYC-36	Yeju tea	*Chrysanthemum mutellinum*	*Chrysanthemum* × *morifolium*	*Chrysanthemum indicum*#	*Chrysanthemum indicum*#
BYC-37	Zhegu tea	*Mallotus sp.*	N	*Mallotus apelta*	*Mallotus sp.*

N, Sequencing without success; Unknown, not identification using DNA barcoding. #Identification results are correct.

plants: 21 kinds of non-*Camellia* teas and 2 kinds of traditional teas (Table 4).

In summary, using one or more DNA data by BLASTN analysis, 23 non-*Camellia* teas were assigned to their original plants successfully.

3.4. *Species identification based on phylogenetic tree*

Some original plants of the non-*Camellia* tea listed on labels lacked GenBank records. So the reference databases only comprised 29 *rbc*L, 26 *mat*K, 22 *psb*A-*trn*H and 29 ITS2 sequences obtained by downloading all the sequences that yielded an *e*-value of 0.0 in the initial BLAST searches, and the measured DNA sequences comprised of 34 *rbc*L, 28 *mat*K, 32 *psb*A-*trn*H and 37 ITS2. All the sequences were also used to construct phylogenetic trees using Mega 5.0 and Clustal X, with a bootstrap value of 1000 replicates, respectively. Moreover, we reconciled preliminary trees by setting the bootstrap value greater than 50% to yield a more credible consensus tree.

In the *rbc*L, *mat*K, *psb*A-*trn*H and ITS2 tree, 13 (39%), 10 (30%), 8 (30%) and 12 (36%) commercialized samples of identification result accord with the original plants of non-*Camellia* tea, respectively (Fig. 1).

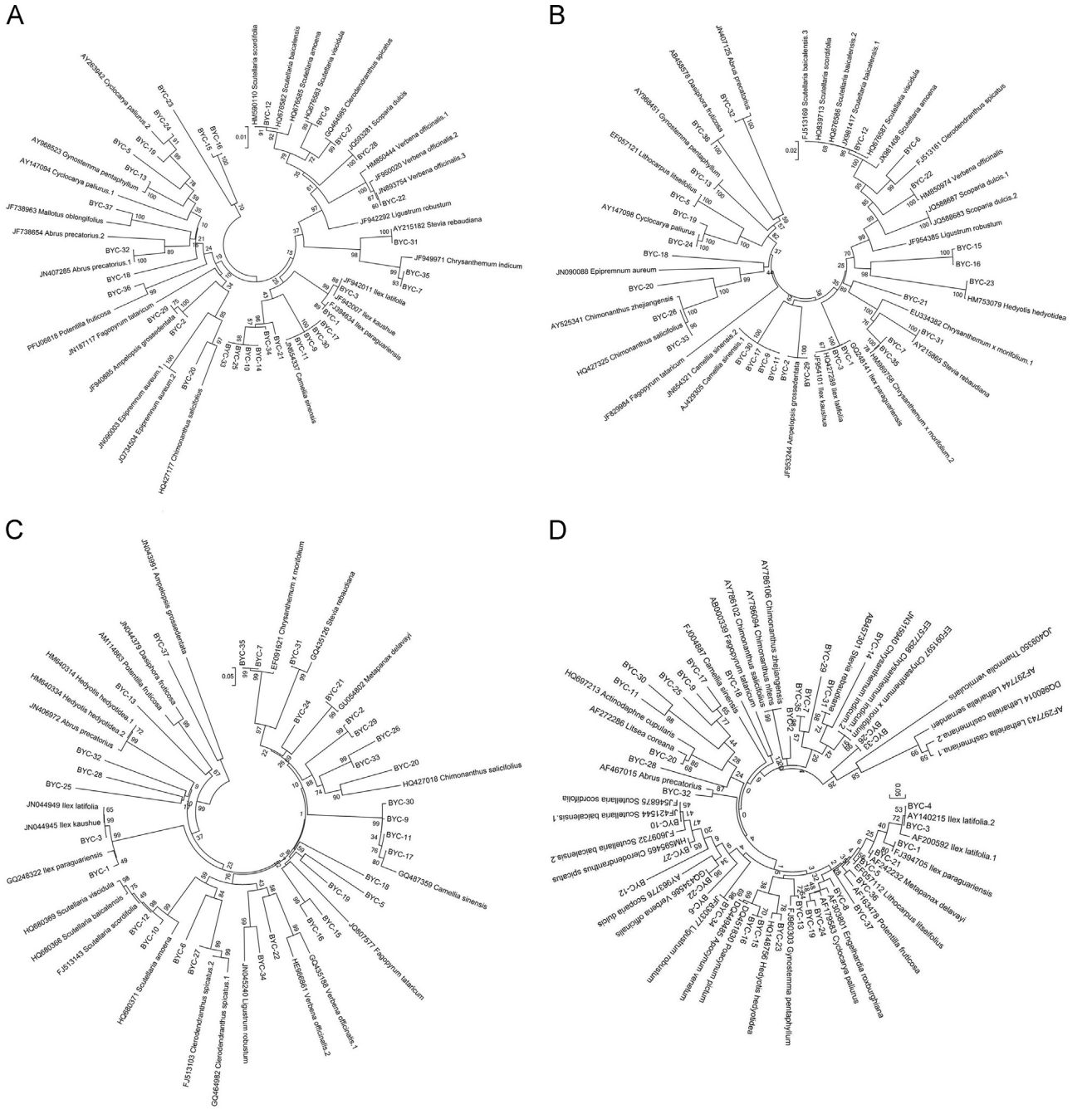

Figure 1 Phylogenetic trees constructed by DNA barcoding sequences. A, *rbc*L; B, *mat*K; C, *psb*A-*trn*H; D, ITS2.

4. Discussion

4.1. Non-Camellia tea of misidentification and potential risk

Non-*Camellia* tea has been widely used in China for centuries. However, correct identification of some of these teas has remained a problem. According to our investigation, some non-*Camellia* teas from several original plants are used wildly in different regions. For instance, Huangqin tea comes from at least four original plants of the *Scutellaria* genus, including *Scutellaria baicalensis*, *S. scordifolia*, *S. amoena*, *S. viscidula,* etc. They were distributed in more than ten provinces in China. However, it is not easy to distinguish differing species of the same genus. Based on experience, the villagers generally seek to collect the herb that has the similar morphological species. Even some people collected other genus species to use as Huangqin tea, such as *Dracocephalum rupestre*. Therefore, it is of great importance to establish an unequivocal identification system for quality control of non-*Camellia* tea for safety and optimum therapeutic use.

4.2. Accuracy of authentication based on BLASTN and phylogenetic tree

In our BLASTN results, 11, 11, 12 and 21 non-*Camellia* teas were identified by *rbc*L, *mat*K, *psb*A-*trn*H and ITS2, respectively. That means the ITS2 have more different loci than chloroplast regions. Of course, the plant species lacking GenBank records should not be ignored.

In all phylogenetic trees, yeju tea, gongju tea and all downloaded species from *Chrysanthemum* were grouped in a clade with strong support, but both samples did not match with their original plants. It means that yeju tea, gongju tea can be identified at genera level by a signal DNA barcoding marker. At the same time, the possibility of mixed-use between yeju tea and gongju tea also should be considered. The identification of genera also existed in *Ilex* (paraguay tea and big leaf kuding tea) in *rbc*L and ITS2 trees. However, additional data are needed for further authentication. *Chimonanthus* (shiliang tea and xiangfeng tea) became more interesting and different because of the diverse plant materials of shiliang tea. Further research with broader sampling of these species will advance the identification work.

Laoying tea is from *Litsea coreana* var. *lanuginose* (Lauraceae) and *Actinodaphne cupularis* (Hemsl.) Gemble (Lauraceae), and the major original plant is the former. In the ITS2 tree, all downloaded sequences of species from Lauraceae were in the same clade with the BYC-20 as sister group, and the commercial sample has much closer relationship with *L. coreana*. This means that laoying tea can be most accurately identified by ITS2.

Finally, some non-*Camellia* teas, such as hongxue tea, baixue tea, lvluohua tea and kuqiao tea, were not accurately matched to their original plants in all trees, indicating significant errors associated with the accuracy of DNA barcording among these species. The limited data in all trees among these species probably contribute to these errors. These results show that commercial non-*Camellia* teas should be identified with more accurate DNA barcoding sequences and broader sampling techniques.

4.3. Safety evaluation based on BLASTN and phylogenetic tree

Consumers have become more interested in the beneficial effects of tea to improve health. However, non-*Camellia* tea is not easily identified by morphological characteristics in the market. Adulteration and misidentification are common in the non-*Camelliaea* teas market, which might be malgenic or lethal. Several kinds of non-*Camelliaea* tea (*e.g.*, *Verbena officinalis* L.) are considered abortifacients, and, if unknowingly consumed by a pregnant woman, could cause miscarriage. Luobuma tea from *Apocynum* (Apocynaceae) also is difficult to morphologically distinguish from some toxic plants in Apocynaceae. In our study, *V. officinalis* L. was accurately identified by DNA barcoding. Luobuhongma tea and luobubaima tea were identified at the genera level by BLASTN and both samples were grouped in a branch separated with other kinds of non-*Camelliaea* tea. However, we did note that DNA barcoding technology can't identify some of non-*Camellia* teas, such as fengwei tea, vine tea and sishi tea. This is because there are only a limited number of these sequences from original plants in GenBank. As a consequence, it is not easy to evaluate the safety of non-*Camelliaea* teas. More original plant sequences need to be submitted to GenBank in order to improve the safety of non-*Camellia* teas

5. Conclusions

Non-*Camellia* tea has been ingested for centuries for cultural and health purposes. These teas have been used to protect health and prevent diseases, such as cancer, hyperlipidemia, hypertension and hyperglycemia. In recent years, with the development and utilization of non-*Camellia* tea, only few non-*Camellia* teas have been developed into beverages, such as jiaogulan tea[78] and kuding tea[79]. But published data concerning the toxicity of some kinds of non-*Camellia* tea are very limited; the pharmacological activity and mechanisms of action for most kinds of non-*Camellia* tea have not been systematically studied. Additional research on all of these aspects of non-*Camellia* tea is needed.

In this study, molecular results revealed that DNA barcoding technology is a viable and effective method to identify non-*Camelliaea* tea. DNA barcoding technology can offer an effective method to help provide more accurate ingredient labels to consumers, thereby helping improve the safety of food and botanicals[80]. This is particularly pertinent in an increasingly global economy where longer and more complex market chains increase distances between suppliers and consumers, and where regulatory agencies are becoming more stringent with food and botanicals[81,82].

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