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The confirmation of *Camellia formosensis* (Theaceae) as an independent species based on DNA sequence analyses

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ABSTRACT. The endemic 'Taiwanese wild tea' represents an invaluable natural resource for the tea industry. The taxon is distributed in the central, southern and eastern regions of Taiwan with populations shrinking dramatically in the past decade. Taxonomically, it has long been treated as a lower taxon either under *Camellia sinensis* var. *sinensis* or *C. sinensis* var. *assamica*. Nevertheless, our recent morphological study indicated that this taxon is different from the other two taxa. In this report, we provide molecular evidence for reassessing its systematic status. Samples representing all the wild populations that are available in Taiwan were sequenced along with samples from the other two taxa at the introns 12-16 and 23 of the *RPB2* gene of nuclear DNA. Phylogenetic analysis recovered a monophyletic group of 'Taiwanese wild tea' well separated from the other taxa. In addition, genetic distances and previous phenetic analyses all supported the identity of this species. A new combination of *C. formosensis* (Masamune *et* Suzuki) M. H. Su, C. F. Hsieh *et* C. H. Tsou is proposed herein.

Keywords: *Camellia formosensis*; Molecular taxonomy; *RPB2* gene; Section *Thea*; Theaceae; Taiwanese wild tea.

INTRODUCTION

'Taiwanese wild tea' has been called the "tea of the gods" by indigenous people. The first written record of this plant was made by Chou (1717). This endemic *Camellia* taxon is an element of broad-leaved forests in the central, southern and eastern regions of Taiwan (Figure 1). Due to its special flavor, 'Taiwanese wild tea' has become an important resource in Taiwan's tea industry. A black tea strain, TTES no. 18, was developed via hybridization between the Taiwanese wild tea as the paternal plant and a cultivar from Burma as the maternal plant and it has become a popular tea item. Despite the economic potential, wild populations of Taiwanese wild tea have drastically declined due to human disturbance. Conservation of the endemic tea is, therefore, urgently required and for this purpose, taxonomical information is essential (Mace, 2004).

Various names have been given to Taiwanese wild tea, indicating the taxonomic difficulties. It was first named *Thea formosensis* Masamune *et* Suzuki (Suzuki, 1937), and later on it was referred to as *Camellia sinensis* (L.) O. Kuntze forma *formosensis* Kitamura (Kitamura, 1950; Hsieh et al., 1996) and *C. sinensis* subsp. *buisanensis* (Sasaki) Lu & Yang (1987). It was even merged with *C. sinensis* var. *sinensis* (Ming, 2000). Such taxonomic uncertainty mostly stems from the high morphological similarities existing among these three taxa. In our previous morphological study, however, the results showed that the Taiwanese wild tea can be well differentiated from other native and cultivated tea taxa by its glabrous ovaries and winter buds (Su et al., 2007). Lai et al. (2001) assessed the genetic relationships of tea cultivars, including the Taiwanese wild tea, based on RAPD and ISSR fingerprinting. All the Taiwanese wild tea samples were clustered together in the phylogenetic trees. Another study using RAPD and AFLP fingerprints also reached the same conclusion (Wachira et al., 2001) though only two samples of the Taiwanese wild tea were included. These studies provide the basis of the tea systematics. Nevertheless, samples of Taiwanese wild tea were too few and plants from its eastern population were not included in either of the phylogenetic studies; meanwhile the inter-relationships among central, eastern and western populations seemed complicated (Su et al., 2007).

Molecular phylogeny based on nucleotide sequences of the *RPB2* gene, a single-copy gene (Denton et al., 1998) encoding the second largest subunit of RNA polymerase,

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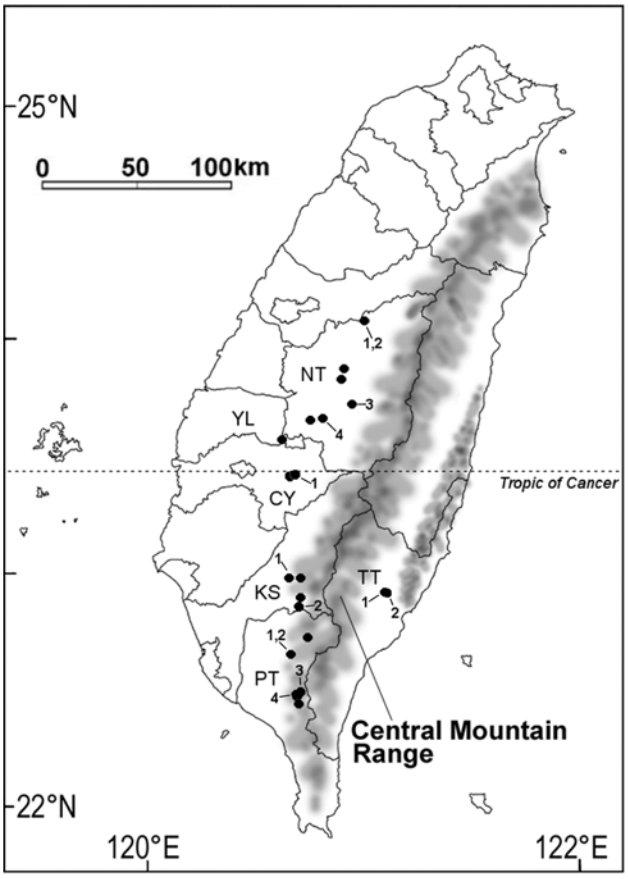

Figure 1. Distribution and sampling sites of *Camellia formosensis*. Solid circles: distribution sites based on specimens of TAI, TAIF, HAST and TNM herbaria. DNA sampling sites are marked with the running number. NT, Nantou County; YL, Yunlin County; CY, Chiayi County; KS, Kaohsiung County; PT, Pingtung County; TT, Taitung County.

provided much insight into the phylogeny of *Camellia* in an earlier studies (Xiao, 2001; Xiao and Parks, 2003); however, no materials from the Taiwanese wild tea were included. We thus reconstructed the phylogeny of the Taiwanese wild tea and its allies by using the same DNA segments, the *RPB2* introns 12-16 and 23, and with a much more extensive sampling. The results are here documented.

MATERIALS AND METHODS

Samples

Thirty-five samples were included in the study, including 13 samples of the Taiwanese wild tea, eight samples of *C. sinensis* var. *sinensis* and 11 samples of *C. sinensis* var. *assamica*, all belonging to the section *Thea* (Table 1). Among the 13 samples of the Taiwanese wild tea, 11 were collected from wild populations (Figure 1) while the other two were obtained from the tea germplasm garden (Table 1). Three species—*C. furfuracea* (sect. *Furfuracea*), *C. transarisanensis* (sect. *Theopsis*), and *C. brevistyla* (sect. *Paracamellia*)—were chosen as outgroups. Leaf materials were preserved in silica gel bags immediately after sampling. Voucher specimens were deposited at the herbaria of TAI or HAST.

DNA extraction, PCR and sequencing

Total genomic DNA was extracted from dried leaf tissue, following the protocol of Vijayan and Tsou (2008). Primers used for PCR amplification and sequencing of the *RPB2* introns 12-16 and 23 are listed in Table 2. PCR was performed in a 50 μL final volume containing 100 ng DNA, 0.2-0.6 μM of both primers, 200 μM of each dNTP, 0.5 U *Taq* polymerase and 1X buffer (Viogene, Inc.). The PCR cycle was with an initial 5 min denaturation at 95°C, followed by 35 cycles of 1 min denaturation at 95°C, 1 min 20 sec annealing at 58°C to 63°C, 1 min 20 sec extension at 72°C, and with a final extension of 7 min at 72 °C. PCR products were purified using QIAquick® PCR Purification Kit (Qiagen Gmbh, Inc.). Automatic sequencing was conducted with an ABI PRISM® 3700 DNA Sequencer.

Data analysis

Sequences were assembled from both directions. Nucleotide sequences of introns 12-16 and 23 were aligned with CLUSTAL_X v1.83 (Thompson et al., 1997). The phylogenetic analyses were performed with the maximum parsimony (MP) and neighbor joining (NJ) methods using PAUP v0.4b10 (Swofford, 2001). In the MP analysis, the most strict consensus tree was obtained by performing a branch-and-bound searching. Indels were treated as single base changes and gaps were considered as the fifth base. In the NJ analysis, distances were measured with an 'uncorrected ("p")' index. Indels were also treated as a single base change but gaps were considered as missing. Branch supports of both phylogenetic trees were obtained by 1000 replicates of bootstrapping. Additionally, the genetic distances between taxa were calculated with the 'uncorrected ("p")' index under the distance criterion of PAUP.

RESULTS

Introns 12-16 of *RPB2* of samples examined were 954 bp long. No indels were detected. The intron 23 was 992 to 1001 bp long in all the samples except for two samples of *C. sinensis* var. *assamica*, i.e., A-Thai-2 and A-Ind-3, in which a long insertion of 284 bp was found. In the analysis, the long insertion was treated as a single mutation and a data matrix of 1923 bp for 35 OTUs was obtained.

Of 1923 bp sequences, 166 bp were variable and 75 bp were parsimoniously informative. Branch-and-bound search found 1896 equally parsimonious trees. The most strict consensus tree was identified with 233 steps. A high consistency index (CI) value 0.92 and low homoplasy index (HI) value 0.25 indicated low levels of homoplasy. MP and NJ trees were the same in topology though the branch supports might be different (Figure 2). All 13 samples of the Taiwanese wild tea formed a distinct

Table 1. List of samples and accession numbers.

Sample code	Voucher no.	Location	Habitat[a]	GenBank accession no.	
				Introns 12-16	Intron 23
C. formosensis					
F-NT-1	Tsou 2133	Meiyuanshan, Taiwan	W	EU849031	EU849066
F-NT-2	Tsou 2135	Meiyuanshan, Taiwan	W	EU849032	EU849067
F-NT-3	Su 683	Derhuasia, Taiwan	G	EU849029	EU849064
F-NT-4	Su 687	Fenhuanshan, Taiwan	G	EU849030	EU849065
F-CY-1	Su 642	Sitin, Taiwan	W	EU849024	EU849059
F-KS-1	Tang 606	Yuyuoshan, Taiwan	W	EU849033	EU849068
F-KS-2	Su 269	Sasi Logging Road, Taiwan	W	EU849021	EU849056
F-PT-1	Su 645	Jenlishan, Taiwan	W	EU849025	EU849060
F-PT-2	Su 647	Jenlishan, Taiwan	W	EU849026	EU849061
F-PT-3	Su 498	Wuweishan, Taiwan	W	EU849022	EU849057
F-PT-4	Su 497	Wuweishan, Taiwan	W	EU849023	EU849058
F-TT-1	Su 655	Yungkangshan, Taiwan	W	EU849028	EU849063
F-TT-2	Su 654	Yungkangshan, Taiwan	W	EU849027	EU849062
C. sinensis var. *assamica*					
A-Bur	Su 669	Burma	G	EU849037	EU849072
A-Chn-1	Wang *s. n.*	Yunnan, China	C	EU849042	EU849077
A-Chn-2	Wang 7740	Zhejiang, China	W	EU849043	EU849078
A-Ind-1	Su 667	Manipur, India	G	EU849036	EU849071
A-Ind-2	Su 670	Assam, India	G	EU849038	EU849073
A-Ind-3	Fatima *s. n.*	Kerala, India	C	EU849044	EU849079
A-Sri	Su 685	Sri Lanka	G	EU849040	EU849075
A-Thai-1	Su 684	Thailand	G	EU849039	EU849074
A-Thai-2	Maxwell 04-212	Lampang, Thailand	C	EU849041	EU849076
A-Tw-1	Su 609	Joefenershan, Taiwan	N	EU849034	EU849069
A-Tw-2	Su 610	Joefenershan, Taiwan	N	EU849035	EU849070
C. sinensis var. *sinensis*					
S-Chn-1	Su 668	Fujian, China	G	EU849048	EU849083
S-Chn-2	Tsou 1957	Guangdong, China	W	EU849050	EU849085
S-Chn-3	Tsou 2052	Fujian, China	W	EU849051	EU849086
S-Chn-4	Tsou 2127	Fujian, China	W	EU849052	EU849087
S-Tw-1	Su 197	Jiantziliao, Taiwan	N	EU849045	EU849080
S-Tw-2	Su 640	Shanmei, Taiwan	C	EU849046	EU849081
S-Tw-3	Su 644	Fonshan, Taiwan	C	EU849047	EU849082
S-Tw-4	Su *s. n.*	Sijhih, Taiwan	C	EU849049	EU849084
C. brevistyla	Su 681	Alishan, Taiwan	W	EU849055	EU849090
C. furfuracea	Su 217	Lienhuachi, Taiwan	W	EU849053	EU849088
C. transarisanensis	Su 515	Yuanchueishan, Taiwan	W	EU849054	EU849089

[a]Habitat: C, cultivated; G, germplasm garden of Yuchih Branch, Taiwan Tea Experiment Station, Taiwan; N, naturalized; W, wild. Samples of *C. formosensis* were indicated in Figure 1 with the running numbers.

clade with high bootstrap values (92 and 99) (Figure 2). Thus, the monophyly of the Taiwanese wild tea was strongly supported. Within this clade, two subclades were recovered that were significantly supported. Samples from southern Taiwan, hereafter defined as the South population, clustered together. Samples from regions of Nantou (defined as the Nantou population) and Taitung (defined as the East population) formed another subclade. In contrast to the monophyly of the Taiwanese wild tea, the 19 samples of *C. sinensis* var. *assamica* and *C. sinensis* var. *sinensis* were polytomous in the cladogram (Figure 2). Since the latter two taxa have a long history of cultivation and hybridization within the tea industry, introgressions between them were expected. Meanwhile, the formation of a distinct clade by all the samples of the Taiwanese wild tea strongly demonstrated that, genetically, the Taiwanese wild tea was well separated from the other two taxa.

The averages of pairwise genetic distances between species and between con-specific populations were shown in Table 3. The average distance between samples of the Taiwanese wild tea and *C. sinensis* var. *assamica* was 0.0092, and that between the Taiwanese wild tea and *C. sinensis* var. *sinensis* was 0.0079. Both distances were about twofold of that between *C. sinensis* var. *sinensis* and *C. sinensis* var. *assamica* (0.0043). Regarding the distance between the different populations of the Taiwanese wild tea, the averages were 0.0016 between the Nantou and the East populations, 0.0038 between the South and

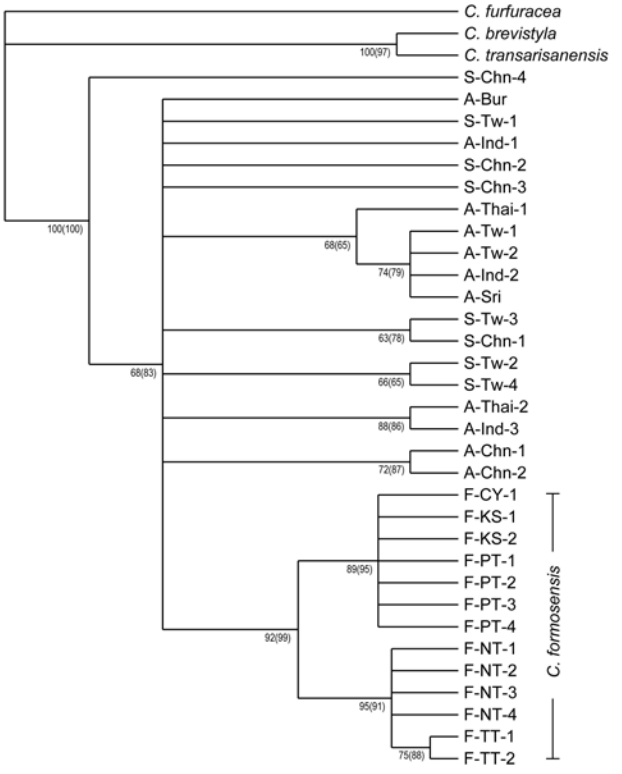

Figure 2. The phylogenetic tree based on sequences of the *RPB2* introns 12-16 and 23. Branch supports of MP and NJ (shown in parentheses) were generated by 1000 replicates of bootstraps.

Table 2. List of primers used in the study.

Name	Sequence (5′-3′)	Target fragment	Source
C-1-11F (forward)	CCACTTATGGGTATCGTCTGGCTG	*RPB2* introns 12-16	Xiao, 2001
C-1-16R (reverse)	GCCTGCTTACCCATTGCTGACTG		Xiao, 2001
C-1-11F2 (forward)	AAGAATCTTGCATTGATGGT		This study
C-1-16R2 (reverse)	ATATGTATTACGTGGGGACT		This study
C-1-10AF (forward)	CCCTCTCGAATGACTATTGG	*RPB2* intron 23	Xiao, 2001
C-1-11R (reverse)	GATAGTATGTGGGACCAAGG		Xiao, 2001
C-1-10AF1 (forward)	GTAAGGTTGCAGCTCACATG		This study
C-1-11R1(reverse)	CCTGTGTGACCATTGTACAT		This study

Table 3. Average genetic distances among *C. formosensis*, *C. sinensis* var. *assamica,* and *C. sinensis* var. *sinensis* and those among the three populations of *C. formosensis*.

	Among taxa		
	C. formosensis | *C. sinensis* var. *assamica*	*C. formosensis* | *C. sinensis* var. *sinensis*	*C. sinensis* var. *assamica* | *C. sinensis* var. *sinensis*
Average genetic distance	0.0092±0.0022	0.0079±0.0026	0.0043±0.0023
	Among populations of *C. formosensis*		
	Nantou population | South population	East population | South population	Nantou population | East population
Average genetic distance	0.0038±0.0002	0.0033±0.0002	0.0016±0.0006

Nantou populations, and 0.0033 between the South and East populations, and these were lower than the average distance between *C. sinensis* var. *sinensis* and *C. sinensis* var. *assamica* (0.0043).

DISCUSSION

The results of the present study demonstrated that Taiwanese wild tea is considerably different from both *C. sinensis* var. *sinensis* and *C. sinensis* var. *assamica*. This is evident from the monophyletic grouping of all the sequences of the Taiwanese wild tea in the cladogram well separated from that of the latter two taxa (Figure 2). This result is consistent with our previous observation on phenetic analysis that all the 52 specimens of the Taiwanese wild tea formed a distinct clade well separated from the 113 specimens of the other two taxa. Furthermore, the pubescences on the ovary and dormant buds along with several other minor characters distinguish the Taiwanese wild tea from the latter two taxa (Su et al., 2007). In the earlier molecular phylogenetic study with *RPB*2 sequences Xiao and Parks (2003) indicated that *C. sinensis* var. *sinensis* was different from *C. sinensis* var. *assamica* and the average of pairwise genetic distance of *RPB2* introns 12-16 and 23 among the species of section *Thea* was 0.0087±0.0054 (Xiao, 2001, Appendix 6 & 7). In this study, the pairwise genetic distances—0.0092, between the Taiwanese wild tea and *C. sinensis* var. *assamica* and, 0.0079, between the Taiwanese wild tea and *C. sinensis* var. *sinensis*—are equivalent to the average species distance of the section *Thea* (Table 3). This clearly suggests that Taiwanese wild tea occupies the position of a distinct species. Recently Vijayan et al. (2009) also found a similar relationship in nr*ITS* sequence analysis and supported the independent species status of Taiwanese wild tea. Therefore, we strongly advocate treating Taiwanese wild tea as an independent species

In the present study, we used three populations separated geographically; hence, the intraspecific variability of the Taiwanese wild tea was also assessed, which provided another interesting point as these three populations showed different relative relationships as suggested by the morphological phenetic study (Su et al. 2007). The present study reveals that the Nantou and the East populations are found closer than each to the South population (Figure 2). The pairwise distances 0.0016, 0.0038, and 0.0033, respectively, between the Nantou and the East, the South and the Nantou, and the South and the East populations, further confirms this relationship (Table 3) although results from phenetic analysis suggested that the Nantou population was closer to the South than to the East population (Su et al., 2007). Such incongruence between the morphological and molecular sequence analysis generally results from convergent evolution or from morphological adaptations to the environment (Wendel and Doyle, 1998). In the present case, the leaves and buds of the East population are hairy while those of the Nantou and the South populations are glabrous (Su et al., 2007) and these traits, *i.e.* leaf and bud pubescences, are the major components that separated the East population from the other two populations in the phenetic trees (Su et al., 2007). It has been shown that morphological traits of vegetative parts are often influenced by the environmental factors (Jonas and Geber, 1999; Santamaria et al., 2003; Ellison et al., 2004), and the climate of eastern Taiwan is in fact quite different from that of central-west and south, especially in winter when the northeast monsoon is prevalent. This event brings strong winds to the Taitung region where the East population locates, but it diminishes when it reaches central-western and southern Taiwan after passing through the Central Mountain Range. Since a windy environment causes high evapotranspiration and leaf hairs are generally thought of as an adaptive structure to reduce evaporation in environments with water stress (Ehleringer, 1982; Woodman and Fernandes, 1991; Kenzo et al., 2008), we, therefore, assume that the pubescences on the bud and leaves of the East population of Taiwanese wild tea evolved as an adaptive trait to cope up with the windy environment. Such divergent evolutions of the East population could be the main factors causing the deviation of the East population in the phenetic tree. Nevertheless, such intraspecific variability within Taiwanese wild tea is much less than the interspecific variability observed among Taiwanese wild tea, *C. sinensis* var. *assamica* and *C. sinensis* var. *sinensis*, which is evident from both Figure 2 and Table 3.

In summary, the results of the present study strongly suggest that 'Taiwanese wild tea' (*C. formosensis*) is distinct from other tea producing taxa, hence, it should be treated as a separate species. The following is the taxonomic treatment of the Taiwanese wild tea as an independent species.

Camellia formosensis (Masamune *et* Suzuki) M. H. Su, C. F. Hsieh *et* C. H. Tsou comb. nov. 台灣山茶

(Figures 3, 4)

Basionym: *Thea formosensis* Masamune *et* Suzuki, S. Suzuki, ed. Taiwan Zyumoku Benran. 262. 1937.—NEOTYPE (designated here): Formosa (Taiwan), Taityu (Nantou Co.), Gyoti (Yuchih), 8 Nov 1935, flowering, *S. Suzuki s. n.* (TAI no. 218078).

Camellia sinensis (L.) O. Kuntze forma *formosensis* Kitamura, Acta Phytotax. Geobot. 14: 59. 1950; Hsieh, Yang & Lin, Fl. Taiwan 2nd. ed. 2: 672-673. 1996.—TYPE: Japan, Kanaya Tea Research Station (Introduced from Taiwan), 21 Nov 1949, flowering, *S. Kitamura s. n.* (holotype: KYO).

A small, evergreen tree, up to 8 m high; trunk up to 40 cm in diameter, usually sprouting from base; bark pale-white, smooth; branches and branchlets glabrous, smooth; winter buds ovate-lanceolate, glabrous or sometimes sparsely pubescent. Leaves alternate, glabrous both sides or sometimes sparsely pubescent beneath, thin-coriaceous, oblong, 7-17 cm long, 2-6.5 cm broad, tip acuminate to cuspidate, base cuneate, margin finely serrate, midrib

elevated on both sides, lateral veins 8-14, usually > 10; petioles 3-10 mm long, glabrous or sometimes sparsely pubescent. Flowers axillary, solitary or 2-4 in fascicles; pedicels cernuous, glabrous, 3-7 mm long; bracts 2, tiny, deciduous; sepals 5, persistent, broadly ovate, 2-3 mm long, 2-3 mm wide, glabrous outside, fully or partly pubescent inside; petals 5-7, white, suborbicular, 7-13 mm long, 6-11 mm wide, glabrous; stamens many, 5-12 mm long, glabrous; ovary 3-locular, glabrous, 2-3 mm long; style 1, 6-12 mm long, usually < 10 mm, glabrous, stigma 3-fid. Fruits globose or depressed globose, 2-3 cm across, glabrous; seeds 1-3, globose or semi-globose, 1 cm across, glabrous, hilium obvoid, 1-2 mm across. Flowering from September to January.

Endemic to Taiwan, in the central, southern and southeastern parts of the island, including Nantou, Yunlin, Chiayi, Kaohsiung, Pingtung and Taitung Counties, at the elevation of 800-1,800 m.

Additional specimens examined: **TAIWAN.** NANTOU CO.: Luku, 7 Oct 1992, *C. C. Liao 719* (HAST); Meiyuenshan, elev. 1,500 m, 12 Jan 1935, *A. Tanimura*

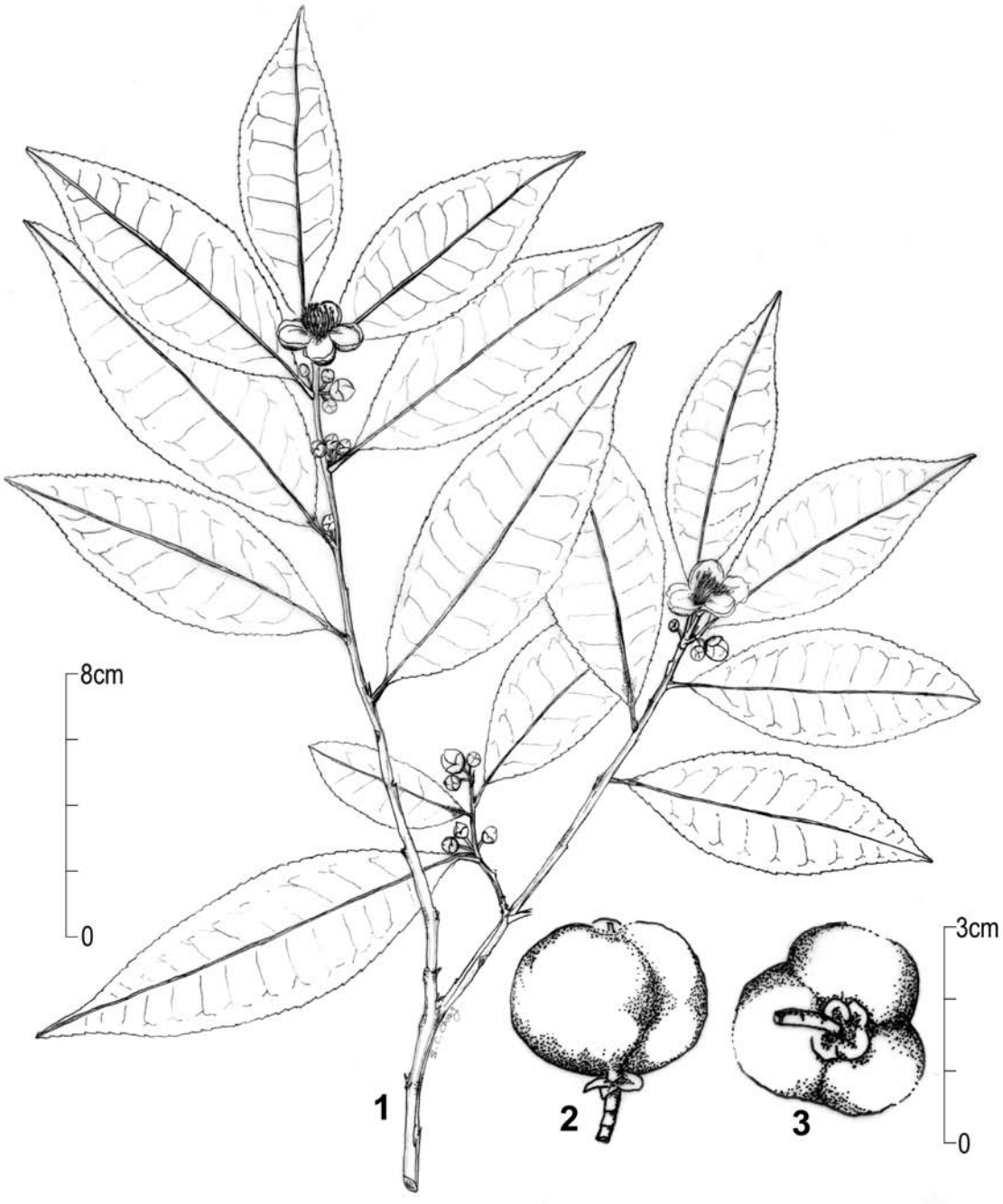

Figure 3. *Camellia formosensis*. 1, flowering branch; 2, mature fruit (side view); 3, mature fruit (bottom view). Left scale bar is for the flowering branch, and right one is for the mature fruit.

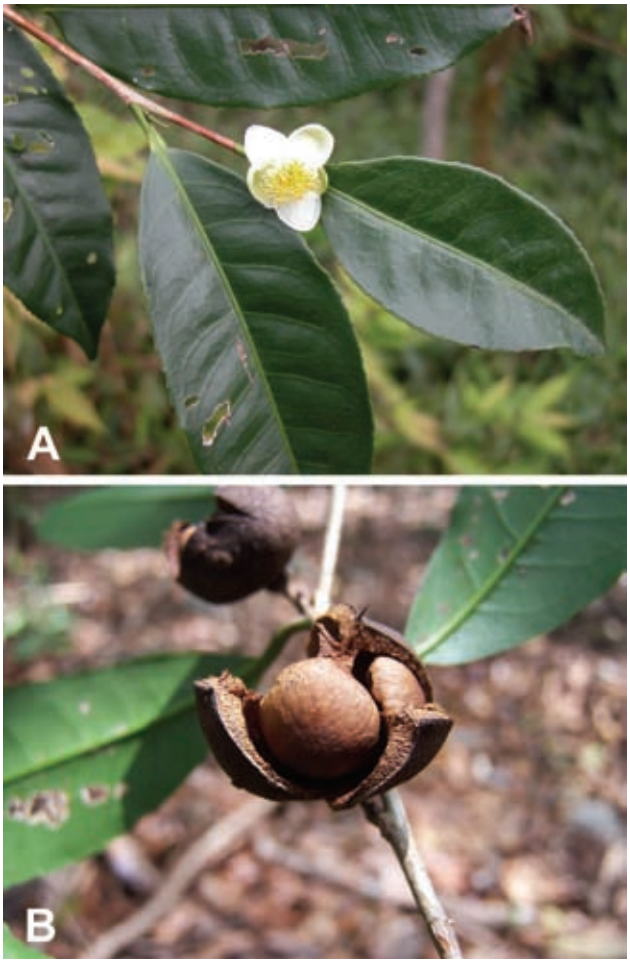

Figure 4. *Camellia formosensis*. A, flowering branch; B, dehiscent fruit.

s. n. (TAI); same loc., 8 Oct 1935, *S. Sasaki s. n.* (TAI); same loc., 8 Nov 1935, *S. Sasaki s. n.* (TAI); same loc., 9 Nov 1935, *S. Suzuki s. n.* (TAI); same loc., 16 Jan 1966, *M. Hasimoto s. n.* (TAI); same loc., 23 Apr 1966, *M. T. Kao 6668, 6676* (TAI); same loc., elev. 1,250 m, 30 Mar 2005, *C. H. Tsou 2132, 2134, 2137, 2139*; Shuili to Yuchih, 25 Dec 1988, *B. J. Wang 15069* (TAIF); Yuchih, 12 Jul 1931, *S. Taniguchi s. n.* (TAI); Yuchih Branch, Tea Research and Extension Station, 13 Jan 1966, *M. Hasimoto s. n.* (TAI); same loc., 15 Nov 2005, *M. H. Su 683, 687* (TAI). YUNLIN CO.: Kulinjiao, 5 Nov 1906, *U. Mori 1901* (TAIF). CHIAYI CO.: Kagi, date unknown, *H. Yamada s. n.* (TAIF); Sitin, 1 Jul 1999, *K. C. Yang 5624* (TNM); same loc., elev. 1,300 m, 12 Sep 2005, *M. H. Su 642* (TAI). KAOHSIUNG CO.: Kakuhozan, 8 Mar 1936. *S. Sasaki s. n.* (TAI); Liukui, 7 Dec 1996, *C. W. Huang 26* (HAST); Nanfong Logging Road, 11 Mar 1986, *S. Y. Lu 18673* (TAIF); Nanfongshan, 8 Feb 1965, elev. 1,350 m, *C. C. Chuang and M. T. Kao 3369* (TAI); Shanping, 10 Dec 1968, *T. C. Huang 4897* (TAI); same loc., 12 May 1971, *T. Kiang and M. T. Kao KT439* (TAI); same loc., 25 Dec 1989, *S. Y. Lu s. n.* (TAIF); same loc., 2 Dec 1996, *Y. H. Lai 83* (TAIF); same loc., 25 May 2004, *C. P. Lin s. n.* (TAIF); Tona, 18 Apr 1986, *S. Y. Lu 18948* (TAIF); Yuyuoshan, 2 Jan 2005, *M. S. Tang et al. 606* (TAI). PINGTUNG CO.: Akohuzi, 7 Nov 1919, *E. Matsuda s. n.* (TAI); Chiupaoshan, 14 Oct 2001, *S. M. Ku 1442* (TAIF); same loc., elev. 1,500 m, 14 Apr 2004, *M. H. Su 575* (TAI); Jenlishan, elev. 1,000-1,100 m, 10 Jul 1993, *J. C. Wang & H. T. Hung 8467* (HAST); same loc., 10 Mar 2004, *M. H. Su 544, 545* (TAI); same loc., 29 May 2004, *S. W. Chung 7090* (TAIF); same loc., 27 Sep 2005, *M. H. Su 645, 646, 647* (TAI); Paiwan, 7 Nov 1912, *E. Matsuda s. n.* (TAI); Sasi Logging Road, elev. 1,300 m, 20 Sep 2003, *M. H. Su 269, 270* (TAI); Wuweishan, 1 Jan 1919, *Matsudai s. n.* (TAIF); same loc., 3 Dec 1995, *K. C. Yang et al. 4527* (TAIF); same loc., elev. 1,000 m, 24 Jan 2004, *M. H. Su 497, 498* (TAI). TAITUNG CO.: Yungkangshan, elev. 900 m, 9 Mar 1986, *S. Y. Lu 18580, 18595* (TAIF); same loc., elev. 900-1,100 m, 28 Sep 2005, *M. H. Su 653, 654, 655, 656, 659, 660, 661* (TAI).

Taxonomic background: the first valid publication of Taiwanese wild tea was by Masamune and Suzuki in 1937 (Suzuki, 1937), in which the plant was named *Thea formosensis,* but no specimens were cited. However, the genus *Thea* had been treated as a synonym of the genus *Camellia* by Sweet (1818), and that was accepted in the International Code of Botanical Nomenclature (ICBN), Article 13.5 (Mcneill et al., 2006). Here, we propose *C. formosensis* as the name of a new combination for Taiwanese wild tea and treat *T. formosensis* as a basionym. In 1950, Kitamura published *C. sinensis* forma *formosensis* and assigned a holotype, but without citing the previous publication of *T. formosensis*. The holotype of *C. sinensis* forma *formosensis* displays the same traits as *T. formosensis*; therefore, *C. sinensis* forma *formosensis* is considered a synonym of *T. formosensis*. Because Masamune and Suzuki (1937) did not cite any specimens in their publication, we now designate one of Suzuki's collections (*S. Suzuki s. n.*, 8 Nov 1935; TAI no. 218078) as the neotype for *T. formosensis* according to the ICBN, Article 9.6 (Mcneill et al., 2006).

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由 DNA 序列分析確認台灣山茶（山茶科）係一獨立的種

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台灣山茶係指台灣特有的一種野生茶樹，是茶樹育種上之重要種原。台灣山茶原生於台灣本島中、南以及東部，但其野生族群正在急速縮減中。分類學上，台灣野生的山茶植物一直被處理為茶 (*Camellia sinensis* var. *sinensis*) 或阿薩姆茶 (*C. sinensis* var. *assamica*) 的變種，不過我們最近形態學上的研究顯示台灣山茶可與上述兩個分類群明顯區隔。在本研究中我們提出分子學上的証據以進一步確認台灣山茶的分類地位。本次樣品涵蓋所有台灣山茶的野生族群以及十九個茶與阿薩姆茶的樣品。我們分析了核 DNA 中 *RPB2* 基因的 introns 12-16 以及 23 二個片段，利用最大簡約法與鄰近連接法來分析，結果顯示分佈於台灣不同地區的野生山茶係一單源的分類群。參考遺傳距離之比較，以及先前所做的數值分類分析，我們確認台灣山茶應處理為一個獨立的種。在此我們提出一個新的組合，將台灣山茶之學名定為 *C. formosensis* (Masamune *et* Suzuki) M. H. Su, C. F. Hsieh *et* C. H. Tsou。

關鍵詞：台灣山茶；茶組；山茶科；*RPB2* 基因；分子分類；台灣野生茶。