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Portable microsatellite primers for *Ficus* (Moraceae)[1]

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- *Premise of the study:* Highly portable microsatellite primers were developed for *Ficus* to facilitate investigation of genetic structure of complete regional floras using a single set of markers.
- *Methods and Results:* Pyrosequencing of five species of *Ficus* produced a library of 5723 potential primers. Potential primers found in at least two species and presenting identical annealing temperatures were tested on a set of five additional *Ficus* species. A set of 20 primer pairs producing well-defined and easily readable peaks was retained and tests showed their potential utility for analyzing population genetic structure of 24 *Ficus* species from Taiwan. Numbers of alleles per locus ranged from one to six in the least variable species and from one to 17 in the most variable species.
- *Conclusions:* The results indicate that our set of primers can be used to analyze polymorphism and compare levels of polymorphism among *Ficus* species.

Key words: coevolution; *Ficus*; Moraceae; mutualism.

The some 800 *Ficus* L. species, along with their mutualistic pollinating wasps and associated communities of nonpollinating fig wasps, constitute an important biological model in which to analyze coevolutionary processes (Cook and Rasplus, 2003). Furthermore, *Ficus* are ubiquitously speciose in tropical lowland rainforests and constitute a major keystone resource for fruit-consuming seed dispersers (Harrison, 2005).

A highly portable set of microsatellite markers was required to produce comparative analyses of the genetic history of regional pools of *Ficus* species. Such analyses should enable us to relate genetic history with: (1) the biological traits of individual *Ficus* species and (2) the structure of associated wasp communities. As such, this set of markers will be a tool for analyses that will provide new insights into our understanding of coevolutionary processes. Analyses using these markers will also help to enhance our understanding of local *Ficus* species diversity.

METHODS AND RESULTS

Five species of *Ficus* representing three subgenera were selected for sequencing: *F. carica* L. of subgenus *Ficus* Corner, *F. microcarpa* L. f. and *F. reflexa* Thunb. of subgenus *Urostigma* (Gasp.) Miq., and *F. septica* Burm. f. and *F. sur* Forssk. of subgenus *Sycomorus* (Gasp.) Miq. Fresh leaves from each species were sampled and dried for three days at 45°C. Genomic DNA isolation was completed using approximately 120 mg of dried leaves of each species and a modified cetyltrimethylammonium bromide (CTAB) procedure (Tel-Zur et al., 1999). For each species, 3–4 µg DNA at a concentration of 200 ng/µL was sent to Genoscreen (Lille, France) for initial microsatellite screening. Enriched microsatellite DNA libraries were produced using an optimized version of the classical biotin-enrichment methods and pyrosequenced (454 GS-FLTitanium pyrosequencing) by Genoscreen according to the methodology described in Malausa et al. (2011). Genoscreen produced, across the five *Ficus* species, a total of 30 103 sequences containing repeated units. A total of 8673 primers were designed, including 5723 primer pairs for sequences containing perfect repeated patterns and 2963 primer pairs for sequences containing compound patterns. Some of these pairs of primers amplified sequences containing the same microsatellite locus when the locus had been recovered from more than one species.

We selected primers that potentially allowed amplification of perfect repeated patterns in at least two species. We imported the 30 103 DNA sequences into a PostgreSQL database (http://www.postgresql.com) and confronted them with the 5723 primer sequences for perfect repeated patterns. Proposed primers that matched the forward and reverse sequences of the flanking regions were selected. We further constrained our choice by selecting for a homogeneous annealing temperature (T_m: 59 ± 1°C), the presence of tri- or dinucleotide repeat units (excluding TA and GC patterns), and a length of at least five repeats. Eighty-six pairs of primers met the requirements and were tested by genotyping six individuals per species for five *Ficus* species belonging to four subgenera (*F. erecta* Thunb. of subgenus *Ficus*, *F. caulocarpa* (Miq.) Miq. and *F. subpisocarpa* Gagnep. of subgenus *Urostigma*, *F. virgata* Reinw. ex Blume of subgenus *Sycidium* (Miq.) Mildbr. & Burret, and *F. benguetensis* Merr. of subgenus *Sycomorus*). PCRs were performed in a 10 µL final volume containing QIAGEN MultiplePCR Mastermix, 2 pmol of each primer, 1 µL of DNA, and DNase-free water. The PCR program consisted of one step of initial denaturation at 94°C (7 min) followed by 35 cycles of denaturation at 94°C (30 s), annealing at 59°C (1 min), extension at 72°C (1 min), and ended with final extension at 72°C for 7 min. Thirty-two of the 86 primer pairs produced clear amplicons of the expected size on agarose gels. These 32 pairs were then tested

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with fluorescent labeling for each forward primer (Applied Biosystems, Villebon-sur-Yvette, France). We chose dyes that allowed for amplification of a minimum of four loci per PCR reaction (Table 1) and performed electrophoresis on an ABI Prism 3130XL 16 capillary sequencer (Applied Biosystems). Across the 32 loci examined, 20 loci were unambiguously readable. We ran the IDT-DNA OligoAnalyzer version 3.1 software to determine which loci could be multiplexed. Identification of loci for multiplex allowed for coamplification of 20 loci in five PCR reactions (Table 1).

Microsatellite amplification was analyzed for a total of 24 of 28 species present in Taiwan. For each sample, 120 mg of dried leaves were placed in a 2 mL plastic tube along with a tungsten bead (diameter 3 mm) and ground in liquid nitrogen at 25 Hz for 1 min with an MM301 tissue grinder (Retsch, Eragny sur Oise, France). DNA extractions were performed using the DNeasy 96 Plant Kit (QIAGEN, Courtaboeuf, France) following manufacturer instructions. PCR amplifications were carried out as described above using our selection of 20 primers. All loci were unambiguously sized using an ABI 3730 sequencer (Applied Biosystems) with the 500 LIZ GeneScan size standard and GENEMAPPER version 4.0 software. Polymorphism was investigated in one population per species on seven species (*F. erecta*, *F. nervosa* B. Heyne ex Roth, *F. virgata*, *F. variegata* Blume, *F. benguetensis*, *F. septica*, *F. subpisocarpa*) for which further genetic studies were planned. All 20 loci amplified and were readable in four of the species, 19 loci were readable in six species, and 17 loci were readable in all seven species (Table 2). Within species, the number of alleles per locus ranged from one to six in the least variable species (*F. benguetensis*), and from one to 17 in the most variable species (*F. virgata*). The number of locally monomorphic loci ranged from one to eight depending on species. We observed presence of microsatellite amplification without scoring polymorphism in the complete set of 24 *Ficus* species from Taiwan (Appendix 1). Out of the set of 20 primers, nine amplified microsatellites in all the species and 14 amplified microsatellites in 21 or more species (Appendix 1).

CONCLUSIONS

Ficus is an old genus originating 60–100 million years ago (Rønsted et al., 2005). Nevertheless, pyrosequencing facilitated independent isolation of identical microsatellite loci in pairs of species from different subgenera. The 20 microsatellite loci identified in this study were highly portable, amplifiable, and easily readable in 24 *Ficus* species belonging to the six subgenera currently recognized within the genus—that is, *Ficus*, *Pharmacosycea* (Miq.) Miq., *Sycidium*, *Sycomorus*, *Synoecia* (Miq.) Miq., and *Urostigma* (Berg and Corner, 2005). We now have a toolbox for a comparative analysis of genetic structure within *Ficus*.

TABLE 1. Characteristics of 20 microsatellite loci in *Ficus*.

Locus	Primer sequences (5′–3′)	Repeat motif	Size range (bp)	5′ dye	Marker set	GenBank accession no.
Car1	F: TCCCTAAACAAGGACAAGGAA R: TATTGCTCATATTGCCAGCG	$(CA)_9$	209–269	6-FAM	1	JN657523
Car2	F: CATCAGAAGAAAAACAAGAACGAA R: TTGGTGTGTTTTGATTTGGC	$(GAA)_6$	187–245	HEX	1	JN657524
Car3	F: GGGGCAACTGTGTTTGAAGT R: TGGCAAGAAACATTATTACCCA	$(TC)_7$	90–143	HEX	1	JN657525
Micr1	F: TATTCCCGAACTGCCAACTC R: AAGTGCCTTGTCAACATCCA	$(GA)_{10}$	78–165	PET	1	JN657526
Car4	F: TTCGACATCGAGAAGGTTCC R: CAAAATGTGCCTTGGGATTT	$(GAA)_5$	94–153	NED	1	JN657527
Car5	F: ATGAACCATGCCTATCGAGC R: TGGAAGCTTGAATGTTTTTGG	$(CT)_8$	66–124	6-FAM	2	JN657528
Car6	F: ACCGCAATTGAGTCTTCACC R: GGTTGCTTGGAATGCAGATT	$(AG)_7$	67–140	PET	2	JN657529
Car7	F: TTTTGGCAGGAAGAAAGGAG R: CTAAATTTGCCGGCCACTAA	$(CTT)_6$	111–251	NED	2	JN657530
Car8s	F: CGGAAGATTTGGATGAGGAG R: TTACAATGGCCACAGCTCAG	$(GAA)_7$	77–135	HEX	2	JN657531
Car9	F: GCGGCAAAATTTCATGGTA R: ACAAAGGGAGCGAGAAGTGA	$(CT)_{10}$	67–162	6-FAM	3	JN657532
Micr2	F: CGAGCAGAAAGATCACAATTCA R: TCAAGTTTTCTGTTGATTCACTCT	$(CA)_6$	81–202	PET	3	JN657533
Sur1	F: TCACCAAATCCAAGTCTCCC R: TTAGTTGCATTTGTGGCACC	$(GA)_8$	137–217	NED	3	JN657534
Car10	F: CTACCGTCCCGGTAATTTCA R: GAGTACCGGCATGATGACCT	$(TG)_7$	97–211	HEX	3	JN657535
Car11	F: TGGTAATGTGGCTGTGAACC R: TAGACTTGCCCTATTGCCCA	$(CA)_8$	160–239	6-FAM	4	JN657536
Micr3	F: TCAACACCAAGAAGCACGAG R: TCTCTTTCTGAACGGTGGCT	$(CT)_{10}$	130–213	PET	4	JN657537
Sur2	F: GACGAACGAGAAGCGAAATC R: GCTTCTCACTCCGTCACGC	$(AG)_8$	125–195	NED	4	JN657538
Car12	F: TTGAAAAACACAAAGTTGATAGCC R: TGTTCACATGCTTAAGTAGGGATATT	$(ATC)_8$	97–180	6-FAM	5	JN657539
Car13	F: TCATGAGCCTAGCAAACGTG R: CCTCAATTCCTTCATTTTTCACA	$(GT)_5$	85–160	HEX	5	JN657540
Car14	F: AGAAACCCTAACGACGGACC R: GGCTGGATTAGATGAATCTGG	$(AG)_5$	88–179	PET	5	JN657541
Sur3	F: AAACTCAGAAACCCTAAACCCC R: TAAAAATAGGGTCGCCGAGA	$(TC)_9$	130–185	NED	5	JN657542

Table 2. Genetic diversity in seven *Ficus* species, one population per species.[a]

		subgen. *Ficus*	subgen. *Pharmacosycea*	subgen. *Sycidium*	subgen. *Sycomorus*			subgen. *Urostigma*
Locus	Genetic parameter	*F. erecta* (N = 61)	*F. nervosa* (N = 21)	*F. virgata* (N = 26)	*F. variegata* (N = 20)	*F. benguetensis* (N = 39)	*F. septica* (N = 30)	*F. subpisocarpa* (N = 26)
Car1	N_a	1*	0	1	4	6	5	1*
	H_o	0	NA	0	0.154	0.571	0.857	0
	H_e	0	NA	0	0.568	0.651	0.684	0
Car2	N_a	2*	1	7	1	2*	2	2*
	H_o	0.300	0	0.750	0	0	0.033	0
	H_e	0.495	0	0.783	0	0.375	0.033	0.500
Car3	N_a	1	0	9	3	5	3	0*
	H_o	0	NA	0.423	0	0.389	0	NA
	H_e	0	NA	0.689	0.593	0.572	0.184	NA
Car4	N_a	1*	7	1	2	2	1	5
	H_o	0	0.714	0	0.059	0.410	0	0.250
	H_e	0	0.837	0	0.057	0.405	0	0.664
Car5	N_a	1	2	3	3	1	2	4
	H_o	0	0	0.267	0.125	0	0.067	0.412
	H_e	0	0.091	0.238	0.633	0	0.064	0.431
Car6	N_a	1	2	3	2	1*	3	2
	H_o	0	1.000	0.563	0.100	0	0.214	0.043
	H_e	0	0.500	0.420	0.095	0	0.254	0.043
Car7	N_a	1*	6	2	5	3	1	4*
	H_o	0	0.714	0	0.688	0.027	0	0.111
	H_e	0	0.749	0.074	0.586	0.053	0	0.562
Car8	N_a	5	2	3	3	2	5	4
	H_o	0.644	0.333	0.480	0.250	0.387	0.241	0.292
	H_e	0.543	0.459	0.486	0.320	0.383	0.278	0.471
Car9	N_a	6	2	8	8	2*	3	11*
	H_o	0.550	0.048	0.154	0.529	0.500	0.633	0.438
	H_e	0.612	0.046	0.681	0.618	0.375	0.543	0.691
Car10	N_a	6	2	5	6	5	3	4
	H_o	0.375	1.000	0.870	0.375	0.150	0.577	0.650
	H_e	0.564	0.500	0.599	0.578	0.473	0.489	0.709
Car11	N_a	1*	4	5	5	4	4	5
	H_o	0	0	0.154	0.250	0.333	0.433	0.423
	H_e	0	0.347	0.595	0.330	0.435	0.473	0.382
Car12	N_a	2*	0	2	3	0*	4*	2*
	H_o	0.846	NA	1.000	0.100	NA	0.143	0.600
	H_e	0.500	NA	0.500	0.265	NA	0.663	0.420
Car13	N_a	1*	3	1	2	1*	3*	0*
	H_o	0	0.050	0	0.125	0	0.400	NA
	H_e	0	0.514	0	0.117	0	0.329	NA
Car14	N_a	1*	3	4	4	2*	9*	2*
	H_o	0	0.952	0	0.813	0.059	0.750	0.059
	H_e	0	0.520	0.694	0.527	0.057	0.679	0.057
Micr1	N_a	9*	6	4	10	5	12	6
	H_o	0.714	0.714	0.423	0.500	0.211	0.680	0.615
	H_e	0.808	0.795	0.514	0.858	0.196	0.797	0.694
Micr2	N_a	2	4	12	4	4	6	9
	H_o	0	0.250	0.654	0.118	0.026	0.700	0.480
	H_e	0.033	0.309	0.845	0.166	0.126	0.702	0.706
Micr3	N_a	7	3	9	2	3	6	4
	H_o	0.772	0	0.125	0	0.103	0.167	0.360
	H_e	0.717	0.503	0.806	0.133	0.402	0.508	0.556
Sur1	N_a	1*	8	17	7	2	6	3
	H_o	0	0.905	0.846	0.765	0.462	0.700	0.040
	H_e	0	0.776	0.902	0.765	0.500	0.716	0.150
Sur2	N_a	3	2	3	3	2*	4*	3
	H_o	0.241	0.250	0.316	0.059	0.059	0.250	0.217
	H_e	0.244	0.289	0.483	0.164	0.057	0.648	0.198
Sur3	N_a	1*	3	4	4	3*	9*	3*
	H_o	0	0.222	0.280	0.188	0.235	0.769	0.200
	H_e	0	0.776	0.458	0.279	0.215	0.825	0.340

Note: H_e = expected heterozygosity; H_o = observed heterozygosity; N = sample size; N_a = number of alleles; NA = not available.

[a] Geographical coordinates for the studied populations: *F. erecta* = 25°11′4″N, 121°31′21″E; *F. nervosa* = 23°54′11″N, 120°52′01″E; *F. virgata* = 22°06′43″N, 120°49′50″E; *F. variegata* = 23°48′35″N, 121°0′0″E; *F. benguetensis* = 23°54′19″N–22°41′8″N, 120°59′17″E–121°35′31″E; *F. septica* = 22°51′17″N, 121°11′43″E; *F. subpisocarpa* = 22°13′45″N–21°55′26″N, 120°48′3″E–120°50′58″E.

* Locus x species combination for which only 20 individuals were genotyped.

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Appendix 1. Results of initial primer screening in 24 *Ficus* species.[a]

	subgen. *Ficus*						subgen. *Pharmacosycea*		subgen. *Sycidium*						subgen. *Sycomorus*			subgen. *Synoecia*		subgen. *Urostigma*			
	sect. *Ficus*				sect. *Eriosycea*		sect. *Oreosycea*		sect. *Sycidium*			sect. *Palaeomorphe*			sect. *Sycomorus*	sect. *Sycocarpus*		sect. *Kissosycea*	sect. *Rhyzocladus*	sect. *Urostigma*		sect. *Conosycea*	
Locus	*F. erecta* (N = 61)	*F. formosana* (N = 5)	*F. pedunculosa* (N = 10)	*F. vaccinioides* (N = 4)	*F. ruficaulis* (N = 2)	*F. triloba* (N = 4)	*F. nervosa* (N = 21)	*F. pubinervis* (N = 6)	*F. ampelas* (N = 10)	*F. cumingii* (N = 2)	*F. irisana* (N = 10)	*F. heteropleura* (N = 4)	*F. tinctoria* (N = 10)	*F. virgata* (N = 26)	*F. variegata* (N = 20)	*F. benguetensis* (N = 39)	*F. septica* (N = 30)	*F. aurantiacea* (N = 3)	*F. pumila* (N = 10)	*F. sarmentosa* (N = 7)	*F. caulocarpa* (N = 10)	*F. subpisocarpa* (N = 26)	*F. benjamina* (N = 10)	*F. microcarpa* (N = 10)
Car1	+	+	+	—	+	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	—	+	+	+
Car2	+	+	+	+	+	—	+	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+
Car3	+	+	+	+	+	+	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	+
Car4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Car5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Car6	+	+	+	+	+	+	+	+	+	—	+	+	+	+	+	+	+	—	+	+	+	+	+	+
Car7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Car8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	+	+	+	+	+
Car9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Car10	+	+	+	+	+	+	+	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+
Car11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Car12	+	—	+	—	—	—	—	+	+	—	+	—	—	+	+	—	+	—	—	—	+	+	—	+
Car13	+	+	+	—	—	—	+	+	+	—	+	—	+	+	+	+	+	—	—	—	—	+	—	—
Car14	+	—	+	—	—	—	+	—	—	—	+	—	+	+	+	+	+	—	—	—	—	+	—	—
Micr1	+	+	—	+	+	+	+	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+
Micr2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Micr3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sur1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sur2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	+	+	+	+	+
Sur3	+	+	+	+	+	+	+	+	—	+	+	+	—	+	+	+	+	—	+	—	—	+	—	+

Note: + = amplified and readable; — = did not amplify or was not readable.

[a] The indicated taxonomic subdivisions of *Ficus* follow Berg and Corner (2005), except for subgenus *Urostigma* for which we follow Corner (1965).

APPENDIX 2. List of voucher specimens used in this study.

No.	Herbarium	Species	Site	Geographical coordinates
*58495	P	*F. microcarpa* L. f.	Chiang Mai University	18°48′28″N, 98°57′16″E
*58458	P	*F. sur* Forssk.	Ithala Game Reserve	27°32′S, 31°18′E
*Ma07-182	P	*F. reflexa* Thunb.	Ranomafana National Park	21°15′28.74″S, 47°25′17.10″E
*FK2009-11	MPU	*F. carica* L.	Vailhauques	43°40′21″N, 3°43′01″E
300Tzeng	TCF	*F. variegata* Blume var. *garciae* (Elmer) Corner	Kenting Forest Recreation Area	21°57′44.67″N, 120°48′37.87″E
301Tzeng	TCF	*F. aurantiacea* Griff. var. *parvifolia* (Corner) Corner	Kenting Forest Recreation Area	21°57′52.75″N, 120°48′40.28″E
308Tzeng	TCF	*F. erecta* Thunb. var. *beecheyana* (Hook. & Arn.) King	Orchid Island	22°00′43.51″N, 121°34′14.15″E
314Tzeng	TCF	*F. vaccinioides* Hemsl. ex King	Kenting Forest Recreation Area	21°57′42.90″N, 120°48′50.76″E
318Tzeng	TCF	*F. virgata* Reinw. ex Blume	Kenting Forest Recreation Area	21°57′52.75″N, 120°48′40.28″E
322Tzeng	TCF	*F. subpisocarpa* Gagnep.	Sheding Nature Park	21°57′20.97″N, 120°49′07.73″E
326Tzeng	TCF	*F. ampelas* Burm. f.	Kenting Forest Recreation Area	21°57′44.89″N, 120°48′37.22″E
330Tzeng	TCF	*F. microcarpa* L. f.	Frog rock, Kenting	21°56′22.21″N, 120°47′55.62″E
333Tzeng	TCF	*F. benjamina* L. var. *bracteata* Corner	Kenting Forest Recreation Area	21°57′47.72″N, 120°48′38.77″E
336Tzeng	TCF	*F. irisana* Elmer	Kenting Forest Recreation Area	21°57′46.69″N, 120°48′44.73″E
340Tzeng	TCF	*F. cumingii* Miq. var. *terminalifolia* (Elmer) Sata	Orchid Island	22°04′49.90″N, 121°33′48.34″E
345Tzeng	TCF	*F. septica* Burm. f.	Sheding Nature Park, Kenting	21°57′18.60″N, 120°49′09.79″E
349Tzeng	TCF	*F. pubinervis* Blume	Orchid Island	22°02′09.72″N, 121°33′32.03″E
350Tzeng	TCF	*F. ruficaulis* Merr.	Orchid Island	22°0′55.33″N, 121°34′18.07″E
352Tzeng	TCF	*F. caulocarpa* (Miq.) Miq.	Kenting Forest Recreation Area	21°57′57.19″N, 120°48′43.86″E
354Tzeng	TCF	*F. pedunculosa* Miq. var. *mearnsii* (Merr.) Corner	Frog Rock, Kenting	21°56′17.26″N, 120°47′56.90″E
366Tzeng	TCF	*F. sarmentosa* Buch.-Ham. ex Sm.	Dongyuan Forest Recreation Area	22°14′37.95″N, 120°50′30.03″E
367Tzeng	TCF	*F. formosana* Maxim.	Kenting Forest Recreation Area	21°57′44.43″N, 120°48′37.17″E
369Tzeng	TCF	*F. heteropleura* Blume	Orchid Island	22°00′58.50″N, 121°34′44.48″E
372Tzeng	TCF	*F. tinctoria* Forst. f. subsp. *swinhoei* (King) Corner	Frog Rock, Kenting	21°56′22.84″N, 120°47′55.53″E
382Tzeng	TCF	*F. triloba* Buch.-Ham. ex Voigt	Dajin Waterfall, Maolin National Scenic Area	22°51′41.62″N, 120°38′43.42″E
386Tzeng	TCF	*F. benguetensis* Merr.	Hushan Reservoir, Yunlin	23°41′40.54″N, 120°37′25.68″E
413Tzeng	TCF	*F. pumila* L. var. *pumila*	Yehliu, New Taipei City	25°12′47″N, 121°41′52″E

Note: MPU = Herbarium of the University of Montpellier 2, Montpellier, France; P = Muséum National d'Histoire Naturelle, Paris, France; TCF = National Chung Hsing University Herbarium, Taichung, Taiwan.

* Specimen used for initial primer screening.