

Supplementary Table 1. List of all the *E. hirta* samples collected from different locations in the Philippines.

Sample No.	Sample Code	Philippine Island Group	Collection Site	GPS coordinates (Latitude, Longitude)	Plate no.
1	Eh01_CAV	Luzon	Mendez, Cavite, PH	14.13161, 120.91784	Eh01
2	Eh02_MUN	Luzon	Muntinlupa City, Metro Manila, PH	14.38512, 121.04626	Eh02
3	Eh03_SAM	Visayas	Sulat, Eastern Samar, PH	11.82105, 125.4124	Eh03
4	Eh04_TAC	Visayas	Tacloban City, Leyte, PH	11.25434, 124.9617	Eh04*
5	Eh05_NE	Luzon	Sta. Rosa, Nueva Ecija, PH	15.45098, 121.05738	Eh05*
6	Eh06_NE	Luzon	Munoz, Nueva Ecija, PH	15.73895, 120.94379	Eh06*
7	Eh07_NE	Luzon	Munoz, Nueva Ecija, PH	15.7295, 120.8729	Eh07*
8	Eh08_QPR	Luzon	Padre Burgos, Quezon Province, PH	13.91893, 121.8449	Eh08*
9	Eh09_PAL	Luzon	El Nido, Palawan, PH	n.d.	Eh09
10	Eh10_QC	Luzon	Quezon City, Metro Manila, PH	14.64471, 121.0525	Eh10
11	Eh11_BTG	Luzon	Ilijan, Batangas, PH	n.d.	Eh11
12	Eh12_BTG	Luzon	Ilijan, Batangas, PH	n.d.	Eh12
13	Eh13_BTG	Luzon	Ilijan, Batangas, PH	n.d.	Eh13
14	Eh14_ANT	Luzon	Antipolo, Rizal, PH	14.59584, 121.18633	Eh14
15	Eh15_ANT	Luzon	Antipolo, Rizal, PH	14.59522, 121.18608	Eh15*
16	Eh16_BIC	Luzon	Albay, Bicol, PH	13.07523, 123.60456	Eh16*
17	Eh17_BAT	Luzon	Pilar, Bataan, PH	14.61623, 120.4962	Eh17
18	Eh18_NVZ	Luzon	Nueva Vizcaya, PH	n.d.	Eh18*
19	Eh19_BUL	Luzon	Plaridel, Bulacan, PH	n.d.	Eh19
20	Eh20_GEN	Mindanao	General Santos, South Cotabato, PH	n.d.	Eh20
21	Eh21_LDN	Mindanao	Iligan City, Lanao del Norte, PH	8.17498, 124.20269	Eh21
22	Eh22_CBN	Luzon	Cabanatuan, Nueva Ecija, PH	n.d.	Eh22
23	Eh23_TAN	Luzon	Tanay, Rizal, PH	n.d.	Eh23
24	Eh24_PAN	Luzon	Urdaneta, Pangasinan, PH	15.97971, 120.60142	Eh24
25	Eh25_BUK	Mindanao	Manolo Fortich, Bukidnon, PH	8.27599, 124.85188	Eh25
26	Eh26_KAL	Luzon	Tabuk, Kalinga, PH	n.d.	Eh26
27	Eh27_CEB	Visayas	Cordova Island, Cebu, PH	n.d.	Eh27
28	Eh28_CEB	Visayas	Sogod, Cebu, PH	n.d.	Eh28
29	Eh29_CEB	Visayas	Consolacion, Cebu, PH	n.d.	Eh29
30	Eh30_CEB	Visayas	Sangi, Mactan Island, PH	n.d.	Eh30
31	Eh31_ILO	Visayas	Iloilo, PH	n.d.	Eh31
32	Eh32_NEG	Visayas	Bacolod, Negros, PH	n.d.	Eh32
33	Eh33_ILC	Luzon	Batac, Ilocos, PH	18.07912, 120.56463	Eh33
34	Eh34_ILC	Luzon	Pasuquin, Ilocos, PH	18.33324, 120.61465	Eh34*
35	Eh35_BIC	Luzon	Albay, Bicol, PH	n.d.	Eh35*
36	Eh36_QC	Luzon	Quezon City, Metro Manila, PH	n.d.	Eh36*
37	Eh37_ILO	Visayas	Iloilo, PH	n.d.	Eh37*
38	Eh38_SUR	Mindanao	Bislig, Surigao, PH	8.17184, 126.23021	Eh38
39	Eh39_DAV	Mindanao	Mati City, Davao Oriental, PH	6.92490, 126.28084	Eh39*

Note: Samples with no GPS coordinates data were labeled “n.d.”. Samples with asterisks in the *Plate no.* column have no voucher specimens. PH = Philippines.

Supplemental table 2. List of sequences used and obtained from this study

Sample code	Gene	Sequence length	GenBank accession no.
Eh01	<i>rbcL</i>	531	MG889511
	<i>matK</i>	863	MG889512
Eh02	<i>rbcL</i>	571	MG889510
	<i>matK</i>	863	MG889513
Eh03	<i>rbcL</i>	568	MG889509
	<i>matK</i>	865	MG889514
Eh04	<i>rbcL</i>	562	MG889508
	<i>matK</i>	898	MG889515
Eh05	<i>rbcL</i>	567	MG889507
	<i>matK</i>	837	MG889516
Eh06	<i>rbcL</i>	457	MG889506
	<i>matK</i>	636	MG889517
Eh08	<i>rbcL</i>	564	MG889505
	<i>matK</i>	831	MG889518
Eh09	<i>rbcL</i>	539	MG889504
	<i>matK</i>	719	MG889519
Eh10	<i>rbcL</i>	568	MG889503
	<i>matK</i>	805	MG889520
Eh13	<i>rbcL</i>	567	MG889502
	<i>matK</i>	864	MG889521
Eh14	<i>rbcL</i>	567	MG889501
	<i>matK</i>	898	MG889522
Eh15	<i>rbcL</i>	566	MG889500
	<i>matK</i>	883	MG889523
Eh17	<i>rbcL</i>	568	MG889499
	<i>matK</i>	882	MG889524
Eh19	<i>rbcL</i>	560	MG889498
	<i>matK</i>	856	MG889525
Eh21	<i>rbcL</i>	558	MG889497
	<i>matK</i>	866	MG889527
Eh23	<i>rbcL</i>	571	MG889496
	<i>matK</i>	810	MG889528
Eh24	<i>rbcL</i>	563	MG889495
	<i>matK</i>	884	MG889529
Eh25	<i>rbcL</i>	564	MG889494
	<i>matK</i>	898	MG889530
Eh26	<i>rbcL</i>	565	MG889493
	<i>matK</i>	898	MG889531

Supplemental table 2 (continued). List of sequences used and obtained from this study

Sample code	Gene	Sequence length	GenBank accession no.
Eh27	<i>rbcL</i>	565	MG889492
	<i>matK</i>	884	MG889532
Eh28	<i>rbcL</i>	561	MG889491
	<i>matK</i>	866	MG889533
Eh29	<i>rbcL</i>	563	MG889490
	<i>matK</i>	863	MG889534
Eh30	<i>rbcL</i>	568	MG889489
	<i>matK</i>	887	MG889535
Eh32	<i>rbcL</i>	563	MG889488
	<i>matK</i>	898	MG889536
Eh33	<i>rbcL</i>	561	MG889487
	<i>matK</i>	871	MG889537
Eh34	<i>rbcL</i>	557	MG889486
	<i>matK</i>	894	MG889538
Eh35	<i>rbcL</i>	558	MG889485
	<i>matK</i>	896	MG889539
Eh36	<i>rbcL</i>	561	MG889484
	<i>matK</i>	898	MG889540
Eh37	<i>rbcL</i>	566	MG889483
	<i>matK</i>	898	
Eh38	<i>rbcL</i>	561	MG889482
	<i>matK</i>	887	MG889541
Eh39	<i>rbcL</i>	562	MG889481
	<i>matK</i>	887	MG889542
Reference <i>E. hirta</i>	<i>rbcL</i>	578	KF432064.1
		703	GU441776.1
		552	JQ591416.1
		662	GQ434078.1
	<i>matK</i>	712	GU441795.1
		1902	HQ645733.1
		1721	MF159450.1
Outgroup (<i>E. hyssopifolia</i>)	<i>rbcL</i>	552	SDH1049-14
	<i>matK</i>	1900	HQ645738.1

Supplemental Table 3. Allele frequencies of the 23 SRAP loci obtained using the PopGene32 software

Locus	Allele 0	Allele 1
E1	0.4201	0.5799
E2	0.3385	0.6165
E3	0.5423	0.4577
E4	0.6417	0.3583
E5	0.0000	1.0000
E6	0.2970	0.7030
E7	0.1715	0.8285
E8	0.8044	0.1956
E9	0.6417	0.3583
E10	0.8044	0.1956
L1	0.5145	0.4855
L2	0.5145	0.4855
L3	0.0000	1.0000
L4	0.8044	0.1956
P1	0.4851	0.5149
P2	0.5688	0.4312
P3	0.4851	0.5149
P4	0.6183	0.3817
P5	0.0000	1.0000
P6	0.7071	0.2929
P7	0.4537	0.5463
P8	0.7670	0.2330
P9	0.8575	0.1425

Criteria: Polymorphic loci is defined as having allele frequencies below 0.99. Thus, E5, L3 and P5 are monomorphic loci.



UP BIOLOGY
Institute of Biology, College of Science
University of the Philippines
Diliman, Quezon City 1101 Philippines



Telephone (632) 981-8500 loc. 3727
Telefax (632) 920-5471

Email: upbiologydiliman@yahoo.com
Website: www.nih.upd.edu.ph

CERTIFICATE OF AUTHENTICATION

This is to certify that the accepted scientific name of the specimen brought by **Francisco M. Heralde III** of **UP Manila Biochemistry** is:

Family Name
Euphorbiaceae

Scientific Name
Euphorbia hirta L.

This specimen was identified using materials at the Institute of Biology Jose Vera Santos Memorial Herbarium (PUH), College of Science, University of the Philippines, Diliman, Quezon City.

This certificate is issued as requested by the aforementioned researcher and may be used for whatever legal matter pertaining and related to this authentication.

Edwino S. Fernando, Ph. D.
Curator
Jose Vera Santos Memorial Herbarium (PUH)

Date: March 14, 2017

Supplemental Figure 1. Plant Authentication of the Voucher Specimens to the Institute of Biology, University of the Philippines Diliman, Quezon City, Philippines.



Supplemental Figure 2. Sample sequencing chromatogram traces. **(A)** An example of poor quality sequences from the Eh_07 *rbcL* amplicons following the sequence chromatogram analysis; it is characterized by too much baseline noises and peaks that are difficult or impossible to call. **(B)** An example of good quality sequence from Eh_03 *rbcL* amplicons following the sequence chromatogram analysis; it is characterized by the absence of baseline noises and easy to call peaks (Q value > 20).

[illegible][illegible]

Supplemental figure 3 (continued). Multiple sequence alignment of *rbcL* and *matK* regions. **(A)** *rbcL* gene alignment of *E. hirta* samples (Eh01_rbcL – Eh39_rbcL), *E. hirta* references (GU441776.1, JQ591416.1, KF432064.1) and *E. hyssopifolia*.

[illegible][illegible]

[illegible]

Eh01_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh02_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh03_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh04_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh05_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh06_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh08_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh09_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh10_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh13_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh14_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh15_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh17_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh19_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh21_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh23_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh24_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh25_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh26_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh27_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh28_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh29_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh30_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh32_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh33_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh34_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh35_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh36_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh37_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh38_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Eh39_rbcL	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
Euphorbia_hyssopifol	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
GU441776.1	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
JQ591416.1	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449
KF432064.1	CCAAAAATTGGGGCTATCCGCTAAGAATTACGGT	AGAGCGGTTTATGAAT	449

(B) *matK* gene region alignment of *E. hirta* samples (Eh01_matK – Eh39_matK), *E. hirta* references (GQ434078.1, GU441795.1, HQ645733.1, MF159450.1) and *E. hyssopifolia*.

Euphorbia_hyssopifol TCTTATTATTCACAGAAATCAATTTCTATTTTCGAAAAAAAAATCCAAGATTTTCTGTGTCCTATATAAATTTTCATA 80
GQ434078.1 TCTTATTATTCACAGAAATCAATTTCTATTTTCGAAAAAAAAATCCAAGATTTTCTGTGTCCTATATAAATTTTCATA 80
GU441795.1 TCTTATTATTCACAGAAATCAATTTCTATTTTCGAAAAAAAAATCCAAGATTTTCTGTGTCCTATATAAATTTTCATA 80
HQ645733.1 TCTTATTATTCACAGAAATCAATTTCTATTTTCGAAAAAAAAATCCAAGATTTTCTGTGTCCTATATAAATTTTCATA 80
MF159450.1 TCTTATTATTCACAGAAATCAATTTCTATTTTCGAAAAAAAAATCCAAGATTTTCTGTGTCCTATATAAATTTTCATA 80

[illegible]

(B) *matK* gene region alignment of *E. hirta* samples (Eh01_matK – Eh39_matK), *E. hirta* references (GQ434078.1, GU441795.1, HQ645733.1, MF159450.1) and *E. hyssopifolia*.

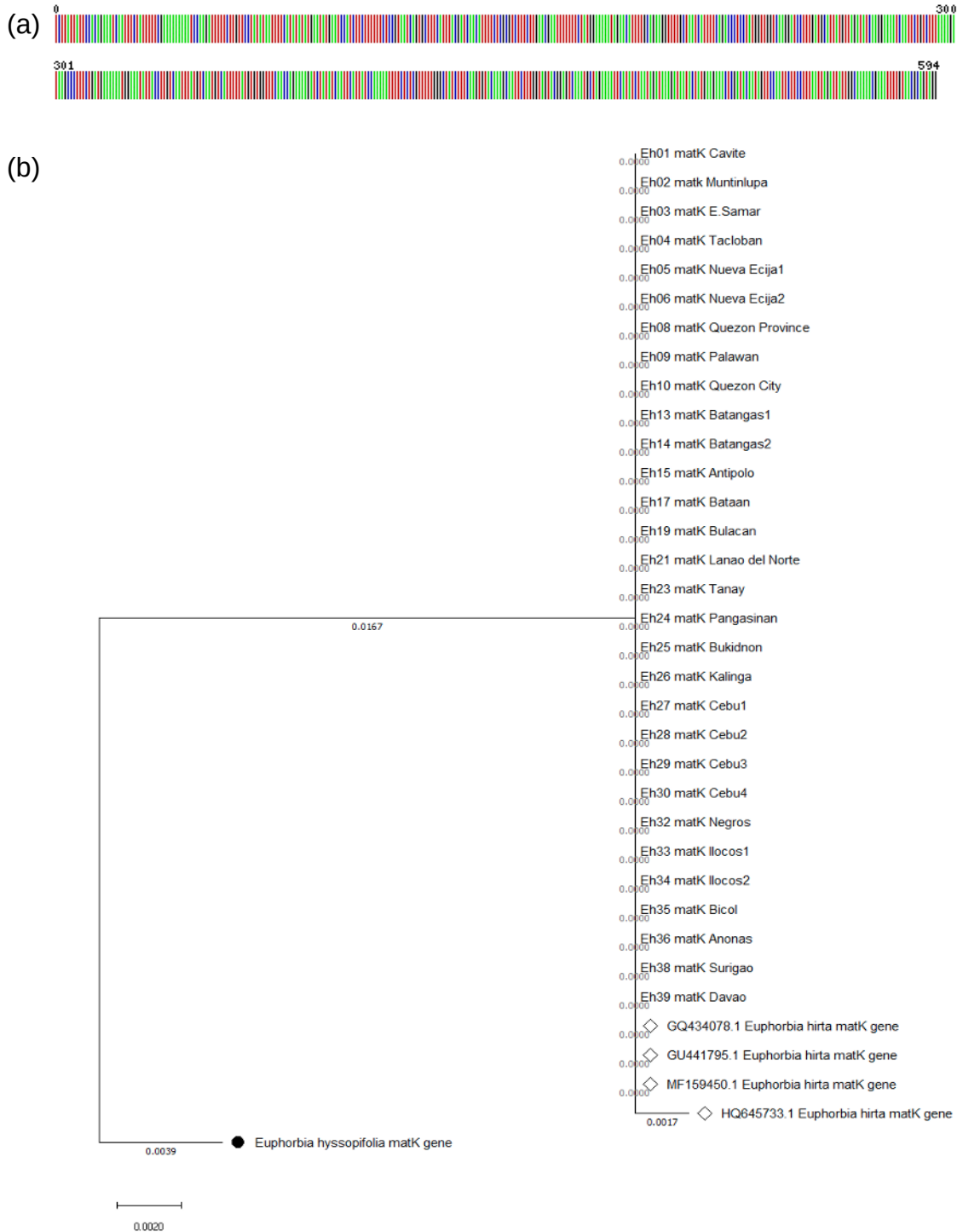
[illegible][illegible]

(B) *matK* gene region alignment of *E. hirta* samples (Eh01_matK – Eh39_matK), *E. hirta* references (GQ434078.1, GU441795.1, HQ645733.1, MF159450.1) and *E. hyssopifolia*.

[illegible][illegible]

[illegible]

Eh01_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh02_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh03_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh04_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh06_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh08_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh09_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh10_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh13_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh14_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh15_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh17_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh19_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh20_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh21_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh23_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh24_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh25_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh26_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh27_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh28_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh29_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh30_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh32_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh33_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh34_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh35_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh36_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh38_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Eh39_matK	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
Euphorbia_hyssopifol	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
GQ434078.1	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
GU441795.1	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
HQ645733.1	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594
MF159450.1	TATTGGCAAAAACGAAATTTTGTAAACGCAGTAGG	594



Supplemental Figure 4. (a) Consensus sequence of *matK* gene with 594 nt from 30 *E. hirta* samples presented as a BOLD illustrative barcode. Bases ACGT are represented by the green, blue, black and red colors, respectively. (b) Maximum Likelihood tree of *E. hirta matK* sequences based on the Tamura 3-parameter model. The bootstrap consensus tree was inferred from 1000 replicates. Name of collection sites are indicated as identifiers and the outgroup sequence is from *Euphorbia hyssopifolia*.



University of the Philippines Manila

RESEARCH ETHICS BOARD

Room 126 NIH Building, University of the Philippines Manila

623 Pedro Gil Street, Ermita, 1000 Manila

Telephone: +63 2 5263-4346; Mobile: +63 927 3264910; Email: upmreb@post.upm.edu.ph

15 August 2024

Mr. Patrick Gabriel G. Moreno, RCh, MSc

Department of Biochemistry and Molecular Biology, College of Medicine, UP Manila

Pedro Gil St., Ermita, Manila City, Metro Manila, 1000

ACKNOWLEDGEMENT OF SUBMISSION

Dear MR. MORENO:

The **University of the Philippines Manila Research Ethics Board (UPMREB)** acknowledges receipt of your completed research manuscript entitled, "**Assessment of Genetic Diversity of *Euphorbia hirta* Linn in the Philippines using sequence-related amplified polymorphism (SRAP) markers**" submitted for UPMREB opinion. This letter serves to inform you that the retroactive review of completed studies that have not previously undergone UPMREB oversight is outside its mandate. However, we can confirm that the research appears to be similar to those that are typically *EXEMPT FROM ETHICAL REVIEW* based on the criteria for exemption in the National Ethical Guidelines for Research Involving Human Participants 2022 (provision 47, page 48), that is, "protocols that neither involve human participants nor identifiable human tissue, biological samples, and data (e.g., meta-analysis protocols)."

A handwritten signature in black ink, appearing to read 'J. Mantaring III'.

DR. JACINTO BLAS V. MANTARING III, MD, MSc

Overall Chair, UP Manila Research Ethics Board