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RESEARCH NOTE

COI-5P Gene Sequence Variation in Philippine *Hydropuntia edulis* (Gracilariaceae, Rhodophyta)

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***Hydropuntia edulis* is an agar producing seaweed. In part due to its economic relevance, the last few decades have seen substantial efforts toward exploring its uses. With these developments, information on its genetic diversity is still, however, incipient. Here, phylogenetic and haplotype analyses in Philippine *H. edulis* were performed based on mtDNA COI-5P gene sequences. Using our newly generated dataset and previous accessions, the range of pairwise intra- and interspecific differences in *H. edulis* was determined. A total of 14 *H. edulis* haplotypes were recovered, of which seven were newly detected. Ten (10) haplotypes were found in the Philippines, eight of which appeared to be exclusive in the region. The genetic relationships in *H. edulis* as inferred from phylogenetic and haplotype network analyses did not follow a clear biogeographic structure. Future work will be necessary to explore the lack of detectable haplotype distribution pattern as presently found. This study can be seen as a fundamental point for future research on the management and conservation of *H. edulis* in the Philippines.**

Keywords: agarophyte, COI-5P, genetic diversity, gracilarioid, haplotype

Gracilarioids are a group of seaweeds valued as an agar source. Like any other seaweed crop commodities, knowledge on their genetic diversity is fundamental for policy decisions related to conservation and use. At the molecular level, phylogenetic and haplotype analyses based on mtDNA COI-5P gene sequences have contributed to assessing the genetic diversity of gracilarioids, especially among the southeast Asian representatives (*e.g.* Yang and Kim 2015).

Hydropuntia edulis (S.G.Gmelin) Gurgel & Fredericq, formerly identified as *Gracilaria edulis* (S.G.Gmelin) P.C.Silva, is one of the most widely distributed gracilarioids in the Indo-Pacific waters (Guiry and Guiry 2020). This alga occurs in a wide range of habitats within the tidal zones but is commonly found on mudflats, entangling among mangroves. As of May 2020, the Web of Science topic search on *H. edulis* (as "*G. edulis*") returned 166 papers, indicating that efforts for the past years involving this taxon have been quite substantial. Studies on DNA sequences of *H. edulis* have been, however, few (Conklin *et al.* 2014; Yen 2014; Yang and Kim 2015) and there appear to be no published genetic diversity studies of *H. edulis* from the Philippines.

Sampling in 38 sites across the Philippines allowed us to collect 61 individuals of *H. edulis* (see Table 1). DNA extraction, PCR, and sequencing followed the procedures outlined in Ferrer *et al.* (2019). Sequence alignment was performed in MEGA ver. 7 (Kumar *et al.* 2016). Phylogenetic analyses were done using maximum likelihood and Bayesian inference *via* CIPRES portal

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Table 1. Collection information for the samples of *H. edulis* used in this study.

Haplotype	Isolate; collection site	GenBank accession number*
Philippine samples		
1	AZE1; Arena Blanco, Zamboanga City	KY995630
1	AZE3; Arena Blanco, Zamboanga City	KY995632
1	BBE1; Binitinan, Balingasag, Misamis Oriental	KY995633
1	BBE2; Binitinan, Balingasag, Misamis Oriental	KY995634
1	DRE1; Damortis, Rosario, La Union	KT357386
1	DSE2; Duyagan, El Salvador, Misamis Oriental	KY995640
1	G325; Bonbon, Oslob, Cebu	JQ026080 [a]
1	2-G320; Currimao, Ilocos Norte	JQ026082 [a]
1	KAE1; Kawas, Alabel, Sarangani	KY995644
1	LBE2; Lucero, Bolinao, Pangasinan	KY995648
1	LLE1; Sitio Reyna Lumaniag Lian	KY995649
1	MSE1; Mapurao, Sta. Ana, Cagayan	KT779924
1	MTE1; Mabua, Tandag, Surigao del Sur	KY995658
1	NLE2; Nalvo Sur, Luna, La Union	KT357385
1	P1CE1; Pob. 1 Calatagan, Batangas	KY995660
1	P1CE2; Pob. 1 Calatagan, Batangas	KY995661
1	P2CE2; Pob. 2 Calatagan, Batangas	KY995663
1	PBE1; Pedro Baculyo, El Salvador, Misamis Oriental	KY995664
1	PBE2; Pedro Baculyo, El Salvador, Misamis Oriental	KY995665
1	POE1; Poblacion Opol, Misamis Oriental	KY995666
1	RBP2; Raw-an Point, Baroy, Lanao Del Norte	KY995667
1	RRE1; Rabon, Rosario, La Union	KT357387
1	SFE1; San Fernando, La Union	KT357382
1	SFE2; San Fernando, La Union	KT357383
1	SGE2; Sapao, Guiuan, Eastern Samar	KY995668
1	SGE3; Sapao, Guiuan, Eastern Samar	KY995669
1	SNE1; Sinakan, Sabtang, Batanes	KY995670
1	SNE2; Sinakan, Sabtang, Batanes	KY995671
1	SVE1; Savidug, Sabtang, Batanes	KY995672
1	TGE1; Taluya, Glan, Sarangani	KY995673
1	TGE2; Taluya, Glan, Sarangani	KY995674
1	TMP1-1; Sta. Teresa, Magsaysay, Occidental Mindoro	KX017511
1	TMP1-2; Sta. Teresa, Magsaysay, Occidental Mindoro	KX017512
1	TMP1-3; Sta. Teresa, Magsaysay, Occidental Mindoro	KX017513
1	TSE1; Talisay, Surigao City, Surigao del Norte	KY995676
1	TSE2; Talisay, Surigao City, Surigao del Norte	KY995677
3	LBE1; Lucero, Bolinao, Pangasinan	KY995647
4	BGE1; Bula, General Santos City	KY995636
4	BSE1; Bato, Sta. Cruz, Davao del Sur	KY995637
4	KGE1; Kapatan, Glan, Sarangani	KY995645
5	BCE1; Burgos, Cortes, Surigao del Sur	KY995635

Haplotype	Isolate; collection site	GenBank accession number*
5	MTE2; Mabua, Tandag, Surigao del Sur	KY995659
6	AZE2; Arena Blanco, Zamboanga City	KY995631
6	G411; Saugali Bay, Zamboanga City	JQ026085[a]
6	LNE1; Ngolos, Guiuan, Eastern Samar	KY995650
6	MIE1; Moro Island, Isabela City, Basilan	KY995651
6	MIE2; Moro Island, Isabela City, Basilan	KY995652
6	MIE3; Moro Island, Isabela City, Basilan	KY995653
6	MRE1; Magsaysay, R.T. Lim, Zamboanga Sibugay	KY995655
6	MRE2; Magsaysay, R.T. Lim, Zamboanga Sibugay	KY995656
7	G323; Bonbon, Oslob, Cebu	JQ026086[a]
7	JJE2; Jampason, Jasaan, Misamis Oriental	KY995643
8	MIP1-1; Moro Island, Isabela City, Basilan	KY995654
9	MRE3; Magsaysay, R.T. Lim, Zamboanga Sibugay	KY995657
12	DPE1; Sitio Diura, Panatayan, Mahatao, Batanes	KY995638
12	DSE1; Duyagan, El Salvador, Misamis Oriental	KY995639
12	HBE1; Hañib, Mahatao, Batanes	KY995641
12	KVE1; Kayvaluganan, Uyugan, Batanes	KY995646
12	PGE1; Sabang, Puerto Galera,Oriental Mindoro	KT779921
12	SBE1; San Miguel, Buenavista, Guimaras	KX017507
12	SBE2; San Miguel, Buenavista, Guimaras	KX017508
12	SSE1; Sabang, Sibunag, Guimaras	KX017505
12	SSE2; Sabang, Sibunag, Guimaras	KX017506
12	THE1; Tuhel, Ivana, Batanes	KY995675
13	HBE2; Hañib, Mahatao, Batanes	KY995642
Extra-Philippine samples		
1	G054; Qingshui Bay, Sanya, Hainan, China	JQ026081[a]
2	859; Tanguisson, Guam	KJ775793[b]
4	G180; Big Korean Island, Penang, Malaysia	JQ026083[a]
4	G167; Big Korean Island, Penang, Malaysia	JQ026084[a]
10	G163; Phuket, Thailand	JQ026088[a]
10	GE0501; Phuket, Thailand	JQ026089[a]
11	G171; Phuket, Thailand	JQ026087[a]
14	GR001; Okinawa, Japan	KF214693[a]
14	GR009; Okinawa, Japan	KF214694[a]

*Accessions without footnote reference were generated by this study; [a]Yang and Kim (2015); [b]Conklin *et al.* (2014)

[Miller *et al.* (2010); see Dumilag (2018) for the detailed parameters].

As is shown in Figure 1, the COI-5P barcoding gap was apparent between *H. edulis* range of intraspecific divergences (0–2.58%) and those of *Hydropuntia* interspecies divergences (5.80–14.97%). Although the level of COI-5P intraspecies variability in *H. edulis* was higher than those reported for other gracilarioids, the computed value still fell within the known ranges for other rhodophytes. For example, the computed values for *Amansia glomerata* C.Agardh and *Asparagopsis taxiformis* (Delile) Trevisan can be as high as 3.6% and 5.3%, respectively [see compilation of intraspecific divergences in Figure 5 of Yang *et al.* (2013)].

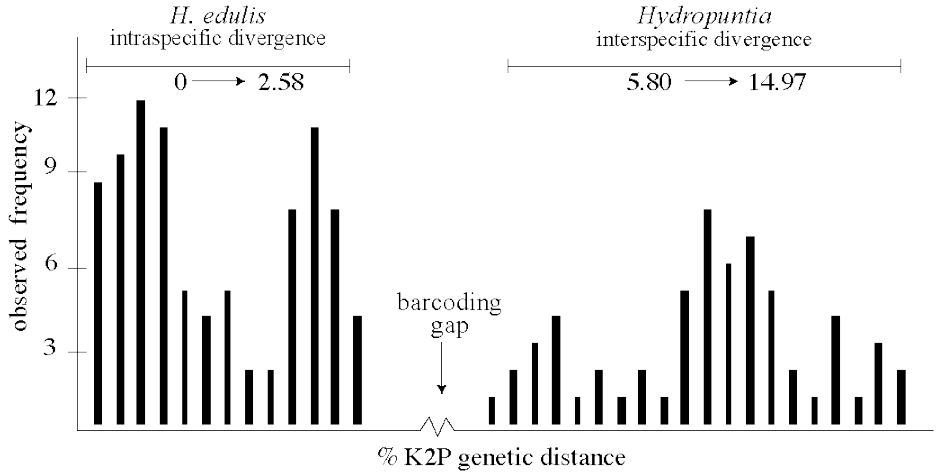

Figure 1. Distribution of pairwise differences in *Hydropuntia* based on COI-5P gene sequences. Low divergence range indicates intraspecific sequence variability among *H. edulis*. Only one sequence per identified haplotype was used. Higher divergence range represents interspecific sequence variability including six (out of eight) currently accepted *Hydropuntia* species: *H. edulis*; *Hydropuntia eucheumatoides* (Harvey) Gurgel & Fredericq; *Hydropuntia multifurcata* (Børgesen) M.J.Wynne; *Hydropuntia perplexa* (K.Byrne & Zuccarello) Conklin, O'Doherty, & A.R.Sherwood; *Hydropuntia preissiana* (Sonder) Gurgel & Fredericq; and *Hydropuntia rangifera* (Kützing) Gurgel & Fredericq.

The maximum likelihood analysis (Figure 2) showed that specimens of Philippine *H. edulis* (n = 65) nested with other conspecific samples. The topology identified the splitting of *H. edulis* into two groups. A larger number of samples (n = 54) from the Philippines and elsewhere were placed in a well-supported group (Group I) while the 11 Philippine and two Japanese specimens formed a separate group (Group II), however unsupported.

Results of the haplotype analysis (Figure 3) indicated two groups within *H. edulis*. The general pattern appeared to match those two groups indicated in the resulting phylogenetic tree. Fourteen (14) haplotypes were recognized. Haplotype H1 had the widest Philippine distribution covering 25 sites extending to Hainan, China. The same haplotype also appeared as the hypothetical ancestral haplotype. In addition to those six haplotypes

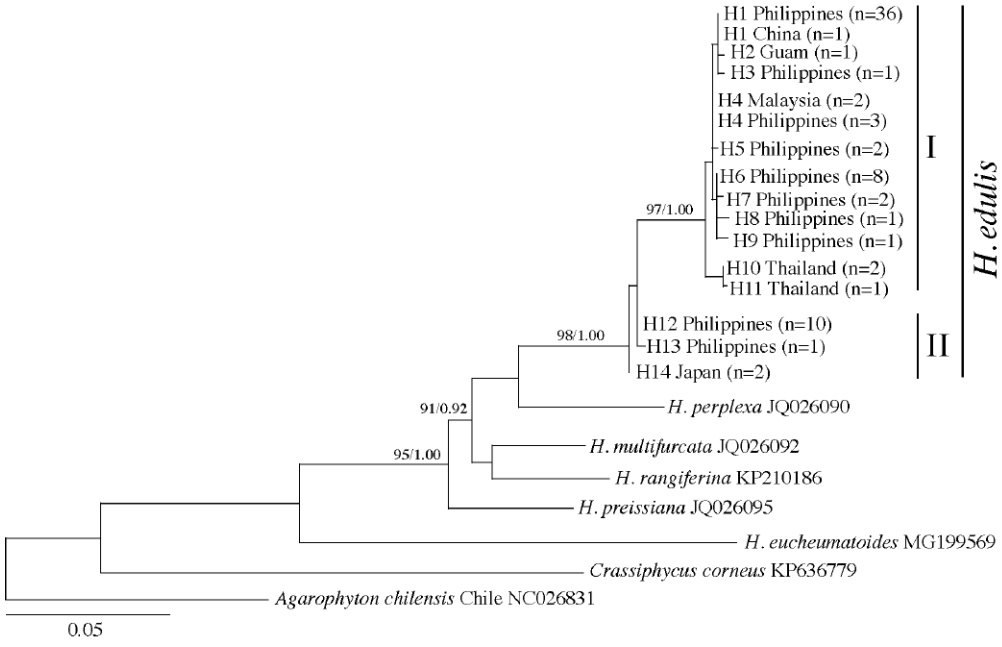

Figure 2. Maximum likelihood tree inferred from COI-5P gene sequences. Values above nodes are bootstrap support (ML) followed by posterior probability (Bayesian inference).

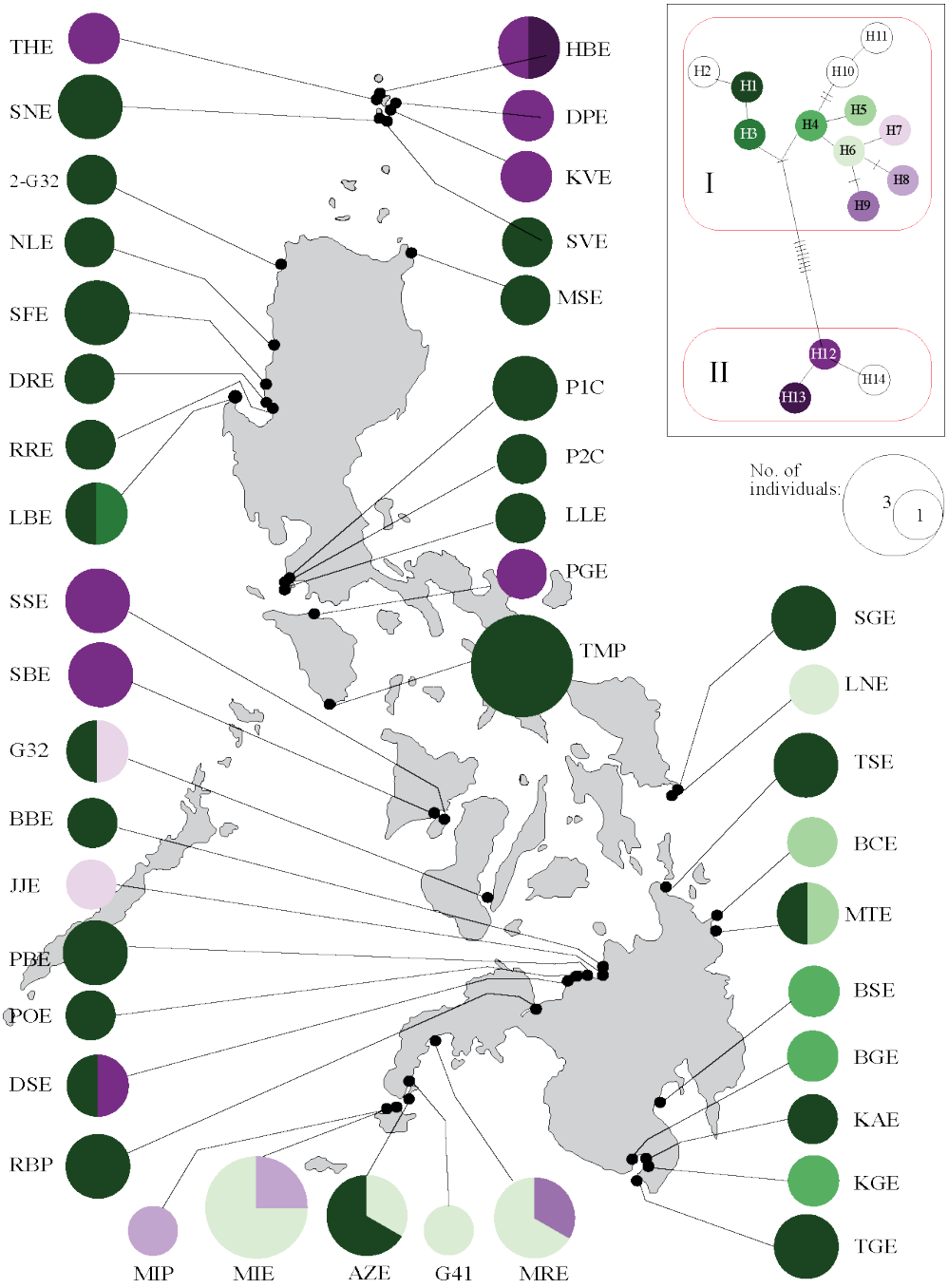

Figure 3. Philippine *H. edulis* COI-5P haplotype distribution and network (A) distribution of *H. edulis* haplotypes in the Philippines. Each pie graph represents the haplotype recovered and number of sequenced individuals per site. (B) *H. edulis* haplotype network using TCS analysis. Line connecting each haplotype (colored circle) indicates a point mutation. A missing haplotype is represented by an open circle. The relative size of each haplotype indicates the number of sequenced individuals.

previously found in Southeast Asian waters, eight novel haplotypes were detected (*i.e.* H3, H5, H6, H7, H8, H9, H12, and H13) – all of which appeared to be exclusive in the Philippines. The biogeographic structure was not observed. The greatest number of recovered haplotypes (n = 8) was from Mindanao, including one shared haplotype (H4) with Malaysian specimens. The Luzon and Visayas region each had only four.

On the basis of COI-5P, gene flow between the nearest populations of *Gracilaria salicornia* (C.Agardh)

E.Y.Dawson within the Philippines has been recently reported (Ferrer *et al.* 2019). A similar case for the Philippine *H. edulis* is, therefore, also expected. Unlike *G. salicornia*, however, there is yet any reported case with regards to *H. edulis* as an introduced species. It is, thus, more likely that those haplotypes that were also detected outside the Philippines may be accounted for their natural distribution. Explicit tests are, however, needed to identify the process that determines their distribution pattern.

Although our survey sites were considerably wide, the number of sequenced samples per site was limited; our current findings might have, therefore, overlooked other occurring *H. edulis* haplotypes. Despite this limitation, our haplotype network indicated exclusive populations of Philippine *H. edulis,* as compared to other neighboring waters. Specimens from the northern Philippines (H12 and H13) were closely related to Japan (H14) while the Malaysian haplotype (H4) was identical to the population sampled from Mindanao. Additional genotyped material collected over a much wider geographic range (*e.g.* Indonesia and Taiwan) are needed to understand the full extent of *H. edulis'* population distribution and differentiation.

In conclusion, we have presented the phylogenetic and haplotype relationships of COI-5P gene sequences of Philippine *H. edulis*. Ranges of intra- and interspecific divergence in *H. edulis* and within the genus *Hydropuntia* were also provided here. This study serves as a reference database that can be used to determine the distribution of various genetically identified populations of *H. edulis* in the Philippines, on which it has implications for resource management and conservation.

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