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TITLE: Genetic species identification and population structure of *Halophila*(Hydrocharitaceae) from the Western Pacific to the Eastern Indian Ocean

AUTHOR: ['Vy X Nguyen', 'Matsapume Detcharoen', 'Piyalap Tuntiprapas', 'U. Soe-Htun', 'Japar B Sidik', 'Muta Z Harah', 'Anchana Prathep', 'Jutta Papenbrock']

ABSTRACT:

The Indo-Pacific region has the largest number of seagrass species worldwide and this region is considered as the origin of the Hydrocharitaceae. *Halophila ovalis* and its closely-related species belonging to the Hydrocharitaceae are well-known as a complex taxonomic challenge mainly due to their high morphological plasticity. The relationship of genetic differentiation and geographic barriers of *H. ovalis* radiation was not much studied in this region. Are there misidentifications between *H. ovalis* and its closely related species? Does any taxonomic uncertainty among different populations of *H. ovalis* persist? Is there any genetic differentiation among populations in the Western Pacific and the Eastern Indian Ocean, which are separated by the Thai-Malay peninsula? Genetic markers can be used to characterize and identify individuals or species and will be used to answer these questions. Phylogenetic analyses of the nuclear ribosomal internal transcribed spacer region based on materials collected from 17 populations in the Western Pacific and the Eastern Indian Ocean showed that some specimens identified as *H. ovalis* belonged to the *H. major* clade, also supported by morphological data. Evolutionary divergence between the two clades is between 0.033 and 0.038, much higher than the evolutionary divergence among *H. ovalis* populations. Eight haplotypes were found; none of the haplotypes from the Western Pacific is found in India and vice versa. Analysis of genetic diversity based on microsatellite analysis revealed that the genetic diversity in the Western Pacific is higher than in the Eastern Indian Ocean. The unrooted neighbor-joining tree among 14 populations from the Western Pacific and the Eastern Indian Ocean showed six groups. The Mantel test results revealed a significant correlation between genetic and geographic distances among populations. Results from band-based and allele frequency-based approaches from Amplified Fragment Length Polymorphism showed that all samples collected from both sides of the Thai-Malay peninsula were clustered into two clades: Gulf of Thailand and Andaman Sea. Our study documented the new records of *H. major* for Malaysia and Myanmar. The study also revealed that the Thai-Malay peninsula is a geographic barrier between *H. ovalis* populations in the Western Pacific and the Eastern Indian Ocean.

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