



Special Issue

***Halophila siaochihkanensis* (Hydrocharitaceae), a new species from Penghu County, Taiwan**Pi-Jen LIU^{1,#}, Chiao-Wen LIN^{2,3,#}, Hsing-Juh LIN^{4,#}, Wei-Jen LIN⁵, Li-Te HSIEH⁶, Cheng-Xuan CAI⁶, Ming-Yang HONG⁷, Pei-Luen LU^{6,*}

1. Graduate Institute of Marine Biology, National Dong Hwa University, 2 Houwan Road, Checheng, Pingtung, 944401, Taiwan. 2. Department of Marine Environment and Engineering, National Sun Yat-sen University, Kaohsiung, Taiwan. 3. The Center for Water Resources Studies, National Sun Yat-sen University, Kaohsiung, Taiwan. 4. Department of Life Sciences at National Chung Hsing University, Taichung, Taiwan. 5. Department of Biological Resources, National Chiayi University, Chiayi, Taiwan. 6. Department of Life Science, National Taitung University, Taitung, Taiwan. 7. Penghu County PengNan High School, Penghu, Taiwan. #Co-first authors, *Corresponding author's email: peiluen@nttu.edu.tw

(Manuscript received 10 December 2024; Accepted 13 March 2025; Online published 27 March 2025)

ABSTRACT: *Halophila siaochihkanensis* P.-L. Lu & P.-J. Liu (Hydrocharitaceae) is described and illustrated as a new species from Penghu County, Taiwan. It is morphologically similar to *Halophila ovalis* (R. Br.) Hook. f. and *Halophila decipiens* Ostenf., but differs in flowering time, leaf hairs, and vegetative life history, having smaller leaves and fewer leaf veins, flowers with white to light yellowish brown tepals, shorter stamens, and fruits that are smaller, globose, and including several seeds in a fruit. This study explores the phylogenetic analysis of 11 species representing all four genera with two outgroup species using DNA Internal transcribed spacer (ITS). The dataset was analyzed using parsimony and maximum likelihood analysis. Results show that *Halophila siaochihkanensis* is monophyletic group. The species is morphologically and phylogenetically distinct from the remaining seagrass species in Taiwan and is recognized as a new species, *Halophila siaochihkanensis* P.-L. Lu & P.-J. Liu, and a new key for seagrass of Taiwan is made.

KEY WORDS: Hydrocharitaceae, *Halophila decipiens*, *Halophila ovalis*, phylogenetics, taxonomy.

INTRODUCTION

The genus *Halophila* Thouars belongs to the family Hydrocharitaceae Juss., and comprises at least 24 described species found across an extensive geographic range, spanning tropical and warm temperate coastlines throughout the Indo-West Pacific region (APG, 2009; Kuo, 2020; WFO, 2024; ColPlantA, 2025). In Taiwan, three species are recognized (Yang *et al.*, 2002; Huang, 2003; Ke, 2004). Most species of this genus are important for ecosystem and biodiversity for marine organisms (Hemminga and Duarte, 2008). Beyond their ecological significance, seagrasses are gaining attention in sociopolitical discussions due to their potential to address the pressing climate and biodiversity crises (Unsworth *et al.*, 2022). Seagrass meadows also contribute to human well-being by supporting fisheries, providing coastal protection, and enhancing water quality through filtration (Unsworth *et al.*, 2022).

The leaf development process in plants of this genus differs from that of most other plants. In typical plants, all parts of the leaf (blade, petiole) grow uniformly from the leaf bud. However, in *Halophila* species, the blade develops first, followed by the growth of the petiole (Ke 2004). The seagrass *Halophila* is the smallest in size among all known seagrasses, yet it is the most diverse group, accounting for over one-quarter of all recognized seagrass species (Den Hartog and Kuo, 2006).

Halophila plants are small and delicate, lacking the

long, strap-like leaf blades characteristic of other seagrasses, making them distinctly unique (Kuo 2020). This unusual morphology has historically complicated their taxonomic placement within monocotyledon classification systems (Den Hartog 1970). The growth locations of *Halophila* plants are dynamic, undergoing annual changes driven by their reproductive and dispersal strategies (Hemminga and Duarte, 2008). Following the fruiting period, the plants produce an abundance of seeds that either remain dormant at their original site or drift with water currents. These seeds germinate and establish new growth in the following spring, contributing to their shifting distribution (Ke, 2004). Moreover, *Halophila* plants exhibit gradual positional changes throughout the year. This movement is a result of stolons extending outward to colonize new areas while older leaves and stolon internodes wither and decay (Kuo, 2020). This natural displacement process is not confined to wild habitats; similar patterns are also observed in artificially cultivated environments, underscoring the genus's adaptability and unique growth behavior (Ke, 2004).

During our floristic exploration of Penghu County in 2024, we encountered an attractive plant with flowers in the intertidal zone of the sandy beach. We collected some individuals for making into specimens and extracted them into DNA library. Morphologically, they are similar to *Halophila ovalis* and *H. decipiens* due to their leaves. After a detailed examination of their morphological characteristics, their closely related species, and phylogenetics, we concluded



Fig. 1. Distribution of *Halophila siaochihkanensis*. The GPS of collection site, GPS: 23° 39' 19.0" N, 119° 34' 51.8" E (23.655780, 119.582693 in google map) in Penghu County, Taiwan.

Table 1: Genebank accession number were used in this study.

Species name	Genebank accession number
<i>Halophila minor</i>	KX668191.1
<i>Halophila decipiens</i>	AF395672.1
<i>Lemna trisulca</i>	PQ437375.1
<i>Lemna minor</i>	OR907745.1
<i>Thalassia hemprichii</i>	PV150657
<i>Zostera japonica</i>	PV156330
<i>Halodule pinifolia</i>	PV156331
<i>Halodule uninervis</i>	PV156332
<i>Halophila beccarii</i>	PV156333
<i>Halophila ovalis</i>	PV156334
<i>Halophila sp.</i>	PV166220

this *Halophila* spp. from Watong, Penghu County, Taiwan, represent a species new to science, below described as *Halophila siaochihkanensis*. This new species is endemic to Taiwan.

MATERIALS AND METHODS

Taxon Sampling

The collection site of *Halophila siaochihkanensis* is shown in Figure 1. A total of 7 seagrass taxa were

examined in this study. One undescribed *Halophila* sp. from Watong and six described *Halophila* seagrass species native to Taiwan were collected. Sources of DNA for sequencing included freshly collected leaves, and the NCBI nucleotide database of *Halophila minor* (KX668191.1), *Halophila decipiens* (AF395672.1), *Lemna trisulca* (PQ437375.1), and *Lemna minor* (OR907745.1). The specimen source, voucher information, locality, and collection number are provided in the Taxonomic Treatment. All novel sequences generated for this study are deposited in GenBank (Table 1). The selection of two outgroup taxa (*Lemna minor* and *Lemna trisulca*) from the Lemnaceae was based on the results of previous studies and were shown to be closely allied to those seagrass genera (Waycott *et al.*, 2007; Tuya *et al.*, 2024).

DNA Extraction and Amplification

Total genomic DNA was extracted from 1.0 g of fresh or 0.2 g of silica gel-dried leaves using the CTAB method (Doyle and Doyle, 1987) with modification (Morden *et al.* 1996). Or after grinding with liquid nitrogen, total genomic DNA was extracted by using the Gene-Spin™ Miniprep Purification Kit (PROTECH, Taipei, Taiwan),

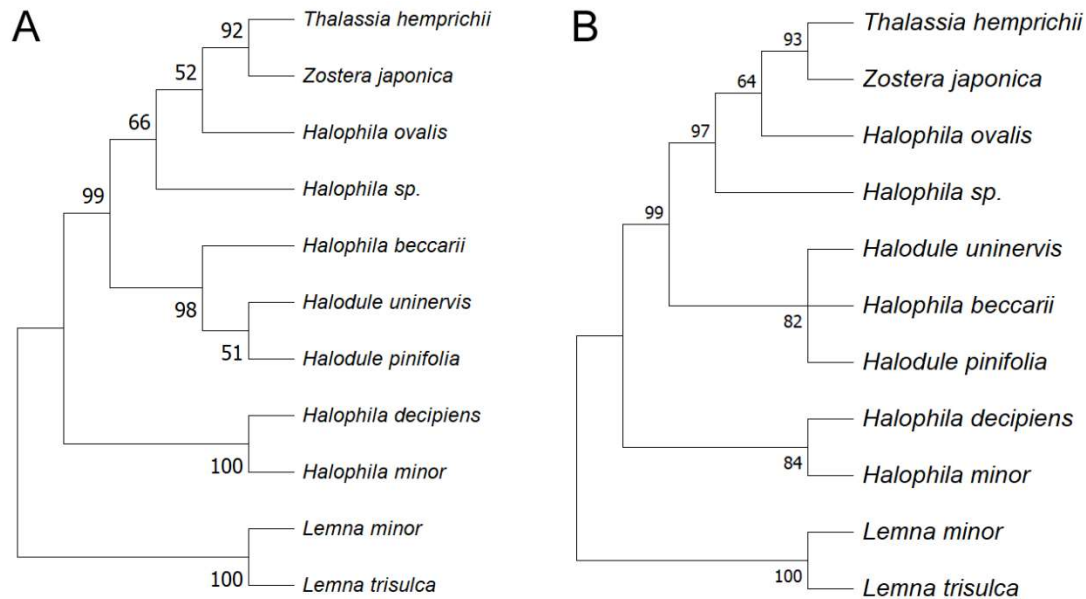


Fig. 2. Phylogenetic trees result from maximum parsimony analysis (A.) and Maximum-likelihood analysis (B.) of the ITS dataset illustrating relationships of species among those seagrass genera, rooted with two closely related non-seagrass members of the Hydrocharitaceae (Tuya et al., 2024). Numbers above the branches represent bootstrap values above 50%. *Halophila sp.* is *Halophila siaochihkanensis*.

and Qiagen DNeasy plant mini kits (Integrated bio, Hsinchu, Taiwan) following manufacturer specifications. DNA samples were accessioned into our Lab plant DNA library.

Species were amplified using standard primers found in the literature (Bogler and Simpson, 1996) based on soybean 18S rDNA, position 1762-1787 (AAGTCGTAACAAGGTTTCCGTAGGTG) and on rice 26S rDNA, position 52-67 (TTTCTTTTCCTCCGCT) with the following PCR conditions: 94°C for 2 min, 35 cycles of 94°C for 30 sec, 50°C for 2 min, 72°C for 2 min, plus a final extension of 72°C for 10 min. Species were also amplified using modified ITS primers (ITS5a: 5'CCTTATCATTTAGAGGAAGGAG-3'; ITS4: 5'-TCCTCCGCTTATTGATATGC-3') found in the literature (Liu and Hsu, 2021). All amplifications were verified on 1.5% agarose gel.

The PCR products were prepared for sequencing using ExoSAP-IT PCR product cleanup protocol of incubation for 37°C for 15 min followed by 80°C for 15 min. Samples were bidirectionally sequenced using each amplification primer at Mission Biotech Ltd (Taipei, Taiwan).

Sequence Alignment and Phylogenetic Analysis

All sequences were aligned initially in Muscle (Edgar, 2004) and then manually adjusted in MEGA 11 (Tamura et al., 2021) following the guidelines of Kelchner (2000) to minimize indels. After the phylogenetic database was constructed, we checked which evolutionary model best fitted the sequences, also applying a partitioning scheme with the concatenated genes. The evolutionary models were tested in the ModelTest-NG package, implemented

in Cypress (Nei and Kumar 2000), limiting the models to those enabled in RAxML and taking the model that best fits the data according to the Akaike information criterion (AIC; Akaike 1974). We obtained that the applicable evolutionary models were the K2 + G (Kimura 2-parameter) for the complete alignment. We performed both maximum parsimony and maximum likelihood (ML) to reconstruct phylogenetic relationships. Maximum parsimony was implemented with MEGA 11 (Tamura et al., 2021). Statistical reliability of the MP tree was evaluated with rapid bootstrapping (1000 iterations) to obtain a MP bootstrap consensus tree with a support threshold $\geq 50\%$. ML inference was implemented with RAxML 8.2.11 (Stamatakis, 2014) in Geneious. Statistical reliability of the ML tree was evaluated with rapid bootstrapping (1000 iterations) to obtain a ML bootstrap consensus tree with a support threshold $\geq 50\%$.

RESULTS

Parsimony Analysis

The Maximum Parsimony (MP) analysis was performed using the combined dataset of all studied genomic regions, yielding 1,953 equally parsimonious trees. The resulting strict consensus tree (Figure 2A) displays bootstrap percentages (BP) exceeding 50% along its branches, providing a measure of support for the inferred phylogenetic relationships.

Within the tree, *Halophila sp.*, identified as *Halophila siaochihkanensis*, is supported as a distinct monophyletic lineage relative to other genera, with a bootstrap value of 66%. This result highlights the unique evolutionary



trajectory of *H. siaochihkanensis* and suggests that it represents a discrete taxonomic entity within the genus.

Although the bootstrap support for the monophyly of *H. siaochihkanensis* is moderate, it still provides valuable insight into its phylogenetic placement. The result aligns with morphological observations and other molecular data, reinforcing its recognition as a new and distinct species. The moderate BP value may reflect challenges such as incomplete lineage sorting, limited resolution from the dataset, or the need for additional molecular markers to improve the robustness of the analysis.

The MP analysis also sheds light on the broader evolutionary relationships within the genus *Halophila*. It reveals a general structure consistent with the results from other phylogenetic methods, such as Maximum Likelihood (ML), though subtle differences in species positioning were noted. These findings underscore the complexity of evolutionary dynamics in *Halophila* and emphasize the importance of using complementary analytical methods to refine our understanding.

Maximum Likelihood Analysis

The Maximum Likelihood (ML) analysis (Figure 2B) of the combined dataset generated a phylogenetic topology that closely resembles the MP (Maximum Parsimony) strict-consensus tree. While the overall relationships among taxa were consistent between the ML and MP analyses, some differences were observed in the positioning of certain species. Despite these variations, the overarching phylogenetic framework remained largely congruent between the two methods.

Notably, the ML analysis provided strong support for the monophyly of *Halophila siaochihkanensis*, which emerged as a distinct and well-supported lineage. This species was confirmed as a monophyletic group relative to other genera in the analysis, with a bootstrap percentage (BP) of 97%, indicating high confidence in its placement.

The strong bootstrap support underscores the robustness of the data and highlights the phylogenetic distinctiveness of *Halophila siaochihkanensis*. This finding aligns with previous morphological and molecular evidence that suggested its unique taxonomic status. Additionally, the similarity between ML and MP topologies strengthens the reliability of the phylogenetic conclusions, emphasizing that *H. siaochihkanensis* occupies a well-defined position within the broader phylogenetic framework of seagrasses. Such results have important implications for understanding the evolutionary relationships within the genus *Halophila* in Taiwan and contribute to a clearer delineation of species boundaries. Future studies could further refine these insights by incorporating larger datasets, additional molecular markers, and samples from broader geographic ranges to test the robustness of these findings across different analytical methods.

TAXONOMICAL TREATMENT

The identification key we developed is primarily based on the foundational work of Yang *et al.* (2002) and Ke (2004), which serve as critical references for this study. In addition to previous publications, we have extensively observed the habitats of these seagrass species across multiple locations over the years to develop this key, including the examination of historical specimens. These publications provided comprehensive descriptions and taxonomic insights that were instrumental in constructing a reliable framework for distinguishing species within the genus *Halophila*.

Yang *et al.* (2002) laid the groundwork by presenting detailed morphological traits, ecological characteristics, and diagnostic features for several seagrass species in Taiwan. Their work emphasized the significance of subtle morphological variations, such as leaf shape, vein patterns, and reproductive structures, which are pivotal for accurate species identification. Similarly, Ke (2004) contributed essential observations on the phenology, growth patterns, and ecological distribution of *Halophila* species, offering a robust dataset that complements the morphological details provided by Yang *et al.* (2002). These insights helped refine the criteria used in our key, ensuring its applicability to a wide range of environmental and geographic contexts. By integrating the findings of these two seminal works, our key aims to provide a practical and precise tool for researchers and conservationists working on Taiwan *Halophila* and, especially for the new species *Halophila siaochihkanensis*. It incorporates morphological traits, reproductive features, phenology, and ecological adaptations, enabling accurate identification even in regions with overlapping species distributions. Additionally, the key is designed to be user-friendly, allowing for efficient identification in both field and laboratory settings.

Key to the species of seagrass in Taiwan

1. Leaves with blade and petiole with well differentiated.
 2. Leaves trinerved at base, lateral nerves absent. *Halophila beccarii*
 2. Leaves penninerved at base, lateral nerves present.
 3. Leaves pubescent on both surfaces, finely spinulose at margins *Halophila decipiens*
 3. Leaves glabrous on both surfaces, entire at margins.
 4. Leaf width less than 4 mm. *Halophila siaochihkanensis*
 4. Leaf width more than 5 mm. *Halophila ovalis*
1. Leaves ribbon-like or linear, petiole and blade similar
 5. Leaves ligulate, parallel longitudinal nerves with fine cross nerves.
 6. Leaves less than 2 mm wide, apex entire, slightly emarginated *Zostera japonica*
 6. Leaves more than 4 mm wide, apex entire, slightly fine-toothed, not emarginated *Thalassia hemprichii*
 5. Leaves not ligulate, parallel longitudinal nerves without fine cross nerves.
 7. Leaf apex obtuse or truncate with irregular teeth.
 - *Halodule pinifolia*
 7. Leaf apex trifid, the later teeth longer than the median, the median tooth obtuse *Halodule uninervis*

**Table 2.** Morphological comparison of *Halophila siaochihkanensis*, *H. ovalis* and *H. decipiens*.

Characters	<i>H. siaochihkanensis</i>	<i>H. ovalis</i>	<i>H. decipiens</i>
Leaf blade	green or reddish, glabrous, elliptic	greenish to brownish green, glabrous, elliptic, oblong, or obovate	bright green, elliptic, puberulous on one or both surfaces
Leaf blade size	4.5–5.5mm in length, 2.5–3.0 mm in width	9–20.2 mm in length, 4.3–8.5 mm in width	10.0–22.0 mm in length, 4.95–6.8 mm in width
pairs of cross-veinlets	5 to 8	12 to 18	6 to 9
Stamens	3	3	3
Anther size	2 mm in length	3.7 mm in length	0.8 mm in length
Stigmata	3	3	3
Stigmata size	2–2.5 cm in length	1.2–1.7 cm in length	4.8 mm in length
Flowering time	March to May	December, April to June, September to October	July to August
Habitat (Water depth)	from 0 to 10 meters	from 0 to 10 meters	from 2 to 40 meters

Halophila siaochihkanensis P.L. Lu & P.J. Liu, *sp. nov.*

小赤炭鹽草 Figs. 3, S1 & S2

Type: Taiwan. Main island of Penghu. Watong (瓦硐), Baisha Township, Penghu County, Taiwan, 23°39'19.0" N, 119°34'51.8"E, 0 m, primaeva subtropical intertidal zone, sandy beach. Plants were collected on April 13, 2024 by P.-L. Lu and L.-T. Hsieh, P.-L. Lu TW1001, deposit in the herbarium of National Museum of Natural Science, TNM (Holotype TNM). Isotypes: TW1002 (TAI)

Diagnosis: *Halophila siaochihkanensis* is morphologically similar to *Halophila ovalis* (R. Br.) Hook. f. in having oval leaf morphology. However, *H. siaochihkanensis* can be clearly distinguished from *H. ovalis* by its small leaves and fewer than 9 pairs branching veins, flowers with pinkish color, smaller flower size, after fruiting, above vegetative parts completely disappearing (Table 2, Figure 3). *Halophila siaochihkanensis* is also morphologically similar to *Halophila decipiens* Ostenf. in having oval leaf morphology. However, *H. siaochihkanensis* can be clearly distinguished from *H. decipiens* by its completely smooth blade, growing elevation above 2 m, small leaves and fewer than 9 pairs branching veins, flowers with pinkish color, smaller flower size, after fruiting, above vegetative parts completely disappearing (Table 2, Figure 3).

Description: Herb, dioecious, growing in sea water, slender and creeping rhizomes with roots emerging from each node, the internodes 3.5–18.0 mm and bear two leaves at each node, leaf scales oval with a transparent, membranous apex. **Leaves** simple and paired. Leaf blades, green or reddish, glabrous, elliptic, 4.5–5.5mm in length, 2.5–3.0 mm in width, margin rounded to obtuse apices, entire, broadly undulating, cross-veinlets 5–8 pairs, often branching. Stems subsessilis. **Petiole** white, slender, spathes elliptic, membranous, transparent, about 3 mm long, not keeled, and shaped like a boat, with acute apices. **Flower** solitary, staminate flowers with three membranous, transparent, cymbiform, tepals approximately 3 mm long, convex when mature and feature a longitudinal dark band. **Anther** basifixed, 2 mm.

Pistillate flowers, solitary and lack tepals. Ovary ovoid, 1.5–2.0 mm long, with marginal placentation, Styles 2.0 cm long, **Stigma** 3, 4.58mm in length.

Etymology: specific epithet is to commemorate the ancestral location. The suggested Chinese name is.

Note: The sediment consists of hard basalt at the bottom, with a layer of shallow to mid-depth sand above. The sand is a mixture of weathered rock fragments and shells, yet it is still classified as a fine sandy beach. The pH value is approximately 7.92–8.59 in winter and 8.47–9.64 in summer. The salinity is around 35 PSU. The dissolved oxygen is approximately 8.22–13.24 mg/L in winter 4.51–6.91mg/L in summer. The plant community in the intertidal zone is purely dominant by this seagrass species. This type of seagrass can be fully exposed during extreme low tides but tends to remain submerged most of the time. There are currently no sources of pollution, and an artificial port is located to the north. The seagrass grows at a certain distance from the artificial fishing port. The sea area is affected by the China Coastal Current, Kuroshio tributaries and South China Sea monsoon currents. Various shellfish, crabs, sea cucumbers, starfish, horseshoe crabs, pufferfish and other creatures are commonly found.

DISCUSSION

The new species of *H. siaochihkanensis*'s flowering season begins in late March and continues through April, with the fruiting period lasting approximately one month. Following this period, the vegetative parts of the plant die back but regenerate by late October, coinciding with the lower sea levels typical of autumn.

In 2024, the population of the newly described species *Halophila siaochihkanensis* in Watong is estimated to exceed 1,000 individuals, yet this species has not been previously documented in *Halophila* publications or specimens. Research indicates that hybridization among *Halophila* species is likely to occur frequently, and notable variations in the leaves of *Halophila ovalis* have been observed in Penghu County. However, many areas

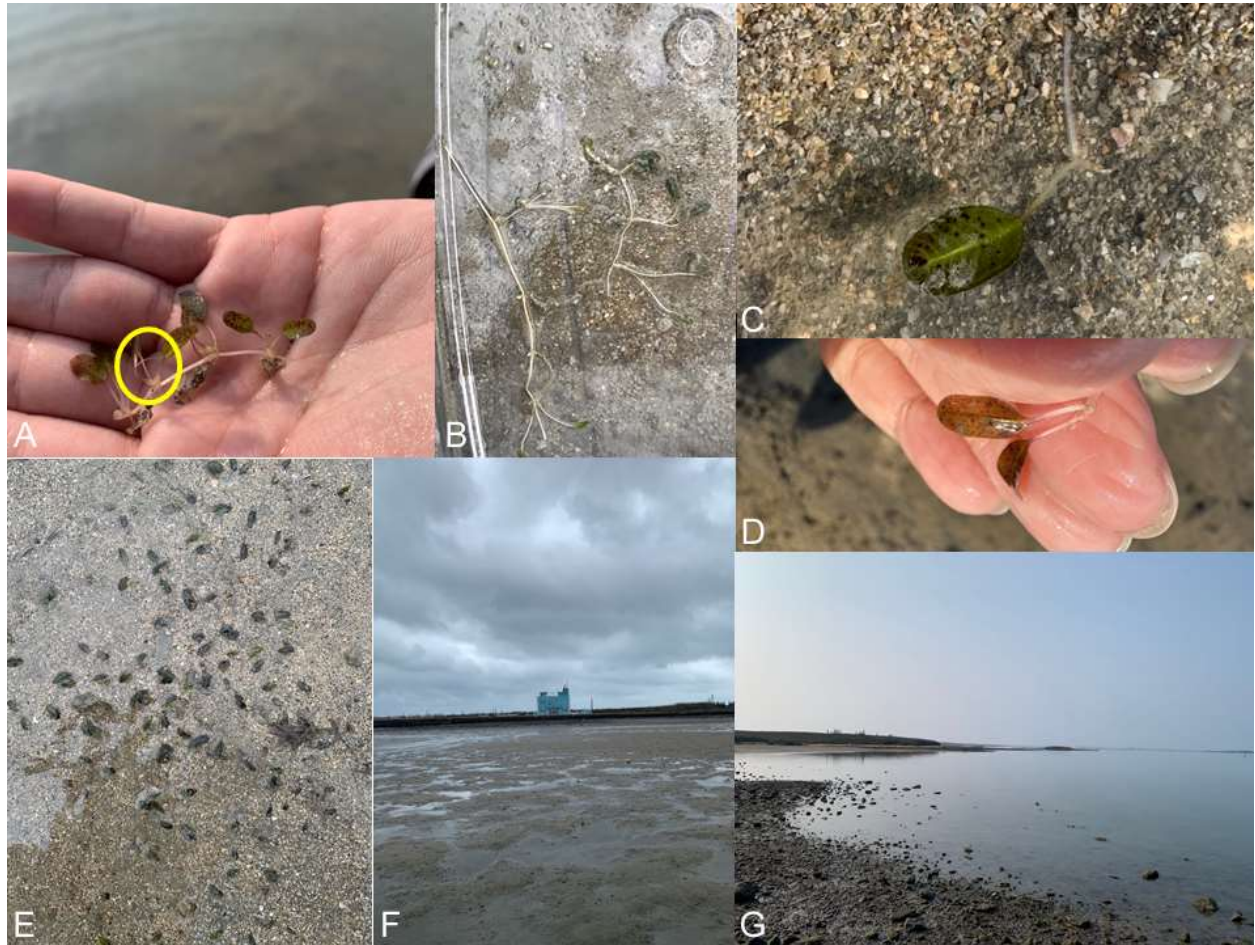


Fig 3. *Halophila siaochihkanensis* P.-L. Lu & P.-J. Liu. The seven pictures indicate the habitat, seagrass bed, and their young/old leave. **A.** The yellow circle indicates the flowers. **B.** The morphology of the individual. **C.** The morphology of mature leaves. **D.** The morphology of young leaves. **E.** The population. **F.** The nature habitat. **G.** The nature habitat.

in Penghu County still lack essential seagrass information. Given the small size of these plants and the limited morphological traits for reliable species identification, we strongly recommend that future molecular studies employ advanced sequencing techniques or develop improved genetic markers. Such methods should analyze multiple samples across the species' geographic range, particularly including specimens from the type locality, to ensure comprehensive and accurate assessments. Consequently, molecular studies and floristic explorations will be vital for understanding not only the coastal areas of Penghu County but also the evolution of *Halophila* and other seagrass species in Taiwan.

According to the International Code of Nomenclature for algae, fungi, and plants (ICN), the description and publication of a new species rely primarily on morphological evidence and the designation of a type specimen. We have provided a well-documented specimen with flowers, which serves as the primary basis for our new species description.

We included *H. minor* samples from the Philippines in our phylogenetic analysis. Our molecular phylogenetic

results remain consistent, showing that the described species does not cluster within the *H. minor* or *H. ovalis*–*H. minor* species complex. The phylogenetic tree clearly supports the distinction of our new species from *H. minor* and *H. ovalis*, reinforcing its recognition as a separate taxon. Given these findings, we believe that our study has sufficiently addressed the necessary comparisons, and that further emphasis on the *H. ovalis*–*H. minor* complex would not alter our main conclusions.

The unique evolutionary trajectory of *H. siaochihkanensis* suggests that this species may be experiencing an 'evolutionary dead end.' While currently viable, its evolutionary pathway may limit its long-term adaptability to future environmental changes, potentially increasing its risk of extinction. This scenario is comparable to certain medical conditions in humans, where a terminal illness has no cure or a severe genetic defect predisposes an individual to future challenges. Although these situations are not mutually exclusive, they illustrate how *H. siaochihkanensis* may represent a lineage that, despite its distinctiveness, faces significant evolutionary constraints.



Further insights into this phenomenon would require a more comprehensive investigation into genome evolution, which is beyond the scope of this study. However, our findings provide a crucial foundation for future research on the evolutionary constraints of *H. siaochihkanensis* and its long-term viability in changing marine environments. While *H. siaochihkanensis* is expected to be endemic to Penghu County, it is at least endemic to Taiwan.

ACKNOWLEDGMENTS

We thank you all people had been involved in this research. Specially appreciate Station Chief of Marine Life Propagation Station, Penghu County, Taiwan, Chih-Hung Ke's supporting in this research, and Mr. Meng-Chi Lee and Bo-Wei Liu's helping in the work. This research was funded by Fisheries Agency, Ministry of Agriculture (TW) grant No 113 -18.3.1-FA-02(1).

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Supplementary materials are available from Journal Website