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# Endophytic Fungi of Emerged Halophytes in River Deltas and Tidal Flats of the Korean Ramsar Wetlands

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**Abstract:** This study aimed to obtain information on the diversity and distribution of the endophytic fungi in Ramsar wetlands. Vast salt marshes in Suncheon Bay, Korea, are formed by two types of ecotones (tidal flats and deltas) that are supported by the emerged halophytes *Phragmites australis* and *Suaeda japonica*. Overall, 324 endophytes were isolated from *P. australis* (six sampling points in the delta and five in the tidal flats) and *S. japonica* (six in tidal flats). Margalef's, Menhinick's, Shannon's, and Simpson's diversity indices significantly varied among the ecotones. In particular, higher variance in diversity value and unevenness was observed in the delta marsh compared with the tidal flat marsh. Further, morphological diversity in the delta salt marsh was 1.8 times higher than that of the tidal flat. Comprising several dominant genera (*Aspergillus*, *Cladosporium*, and *Penicillium*), *Epicoccum*, *Paraconiothyrium*, *Septoriella*, and *Talaromyces* were widely distributed regardless of the aquatic conditions or halophyte species. This study highlights the distinct distribution and diversity of marine endophytes in various ecotones that can physically protect the coastal areas. This data is of much value to secure a national culture collection for future restoration of the coastal wetlands and their ecosystems.

**Keywords:** halophyte; marine endophyte; *Phragmites australis*; Ramsar wetland; *Suaeda japonica*



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## 1. Introduction

Salt marshes (coastal wetlands), transitional zones between land and ocean, are areas in which water is permanently collected, thereby constituting a biodiversity repository [1,2]. One of the origins of salt marshes is tidal flats in coastal areas, a type of ecotone between land and ocean. They develop well on coasts protected by bays or islands, being exposed during low tide and submerged below sea level during high tide [2]. Salt marshes also develop in estuaries at river mouths where freshwater, seawater, and land meet [1]. As the water flow rate slows down in estuaries, inorganic and organic matter form sedimentary sites. In turn, condensed flourishing of emerged halophytes in those sites creates structurally stable salt marshes or coastal wetlands [2,3].

However, because salt marshes are structurally stable, they can be easily depleted by the construction of seawalls. For example, salt marshes on the Korean Peninsula have quickly disappeared [4,5], with 25% of tidal flats and coastal wetlands having disappeared within 50 years, while the destruction of the remaining region is ongoing [4,5]. After the 21st century, owing to preservation efforts, 23 Ramsar wetlands were secured [4,5], with the most representative salt marsh being Suncheon Bay. Because tidal flats surrounding the bay have developed and deltas have formed in the estuary, these two types of ecotones

are a valuable repository of vast animal and microbial biodiversity. In addition to the environmental effect of tidal ranges unique to tidal flats, severe freshwater flow from the large river represents an additional physical stress, leading to the dominance of certain emerged halophytes that can withstand the water flow rate. Due to the advantage of these emerged halophytes, huge deltas and tidal flat salt marshes are physically supported and are still expanding in Suncheon Bay [4,5].

The emerged plants *Phragmites australis* and *Suaeda japonica* form a highly dominant colony with high density, a complex environmental feature that differs from other salt marshes in the Korean Peninsula. In addition, the emerged halophyte species, which survive and thrive in this complex and ever-changing environment, have a differentiated ecology from the coastal plants growing on simple coastal sandy beaches. The salt marshes developed in river estuaries and tidal flats, as well as their *P. australis* and *S. japonica* populations native to Suncheon Bay, serve as a natural purification system, as the potential habitat of various higher life forms, and also as a supporter or extender of the natural salt marshes. Therefore, the ecology of these two emerged halophyte species hold important academic and practical value for future restoration of other disappearing coastal wetlands.

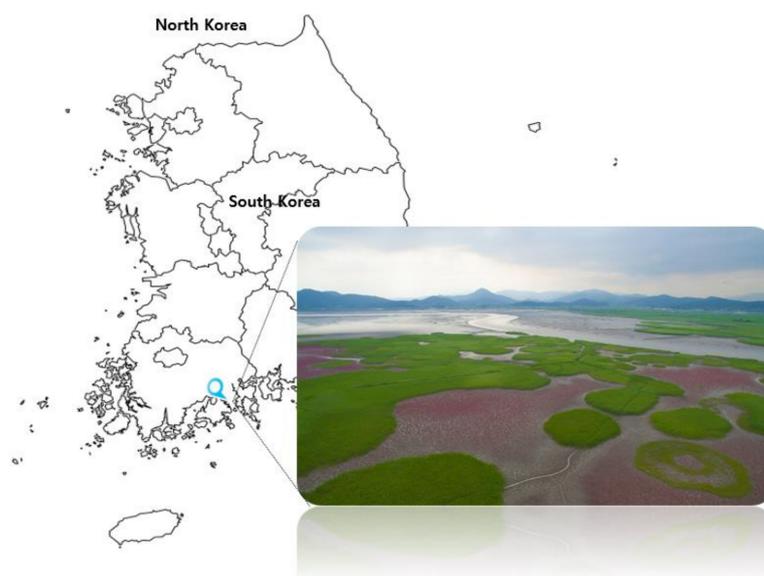
Endophytic microorganisms play key roles in plant growth and reproduction, and can also induce resistance to harsh environments, such as extreme marine conditions [6–8]. Therefore, securing the culture collection of endophytic fungi native to emerged halophyte species and identifying their ecological diversity at well-preserved sites are essential for restoring salt marshes in the future. Such a culture collection representative of the biodiversity of these areas would be of great value in light of the Nagoya Protocol and to secure the availability of biological resources [9,10].

In this study, molecular identification of the endophytic fungi within *P. australis* and *S. japonica* species was conducted using the signature sequences of 324 fungi isolated based on their morphological features. Their distribution and diversity per salt marsh and per plant species was analyzed, and their phylogenetic relationships were derived. Overall, this study aimed to assess the differences among salt marshes developed in deltas and tidal flats, and how these affect the characteristics of endophytic clusters in the well-conserved Ramsar wetlands in the Korean Republic.

## 2. Materials and Methods

### 2.1. Site Description

The salt marshes of Suncheon Bay reflect the vast, complex, and diverse aquatic conditions of the Ramsar wetlands, located on the southern coast of the Korean Peninsula (Figure 1).



**Figure 1.** Location of Suncheon Bay and detailed view of the highly dominant status of two emerged halophyte species: *Phragmites australis* (green colony), *Suaeda japonica* (reddish-purple colony). Map was adapted from the Cultural Heritage Administration of Korea website [11], with permission from the Cultural Heritage Administration of Korea, 2020.

The designated area of the Ramsar wetlands covers salt marshes formed around the deltas of river estuaries (Figure 2A), where freshwater and seawater meet (a transitional zone) [12,13], and in tidal flats of the long beach, developed on the left and right of the inner side of the bay (Figure 2B,C). These ecotones not only contain species common to the communities on both sides, but also include several highly adaptable species that tend to colonize such transitional areas [13]. These two types of salt marshes that have developed in Suncheon Bay show saturated natural soil conditions without water drainage and with the underground water level approaching the surface [14]. According to soil texture classification, salt marshes have a fine clay layer of more than 25 m above the ground, and the area of the tidal flat is approximately 264,462 m<sup>2</sup>. A total of 25 genera and 33 halophyte or coastal plant species have been identified; however, shrubby sea blite (*S. japonica*) and common reed (*P. australis*) are the two dominant species in this area (Figure 1), due to the strong selective pressure of the ecotones. Other coastal or halophyte plants species inhabit the sand on the beaches from the tidal flats and deltas, but only *P. australis* and *S. japonica* serve to preserve the tidal flats and river estuaries.

## 2.2. Plant Sampling

Two emerged halophytic plant species, namely *P. australis* and *S. japonica*, were sampled from the tidal flats that are formed in two large areas south of the main river estuary (Figure 2B,C), and the estuary delta marsh that is formed by the accumulation of organic minerals in the opposite direction of the river bend (Figure 2A).

These three areas (Figure 2) represent the salt marsh geology that is strongly maintained by *P. australis* and *S. japonica* colonies for long time periods. Moreover, these three areas are continuously expanding due to this vegetation. Therefore, two emerged halophyte vegetations supporting the wetland terrain were sampled from the delta salt marsh (six sampling sites for *P. australis*) and tidal flats (five and six sampling sites for *P. australis* and *S. japonica*, respectively) (Table 1 and Figure 2). The total number of sampled sites was 17 across three regions. All of these colonies were collected from sites approximately 100 m apart from each other to ensure ecological segregation (Table 1). The roots of the halophyte species were sampled, followed by the harvest of well-flourished and conserved communities (15 individuals per each species), along with the collection of proximal root layer beach soils from their habitats to minimize physiological changes.



**Figure 2.** Aerial view of each sampling site (indicated in yellow). Salt marshes developed in (A) the delta of Suncheon Bay (*Phragmites australis* colonies at six sampling sites), (B) tidal flats (five sites of *P. australis* colonies), and (C) tidal flats (six sites of *Suaeda japonica* colonies). The aerial images were collected using Google Earth (version 7.3.3) and modified for this study.

**Table 1.** Description of the sampling sites at each salt marsh and endophytic culture collection.

Host Species	Sampling Sites	GPS Coordinates	Fungal Isolates and Taxon						
			Total Isolate Number	Division	Class	Order	Family	Genus	
<i>Phragmites australis</i>	Delta	1	N 34°53'00.20" / E 127°30'49.60"	25	1	4	8	14	18
		2	N 34°52'55.78" / E 127°30'50.43"	25	1	4	7	11	17
		3	N 34°52'54.36" / E 127°30'53.09"	36	3	6	8	13	16
		4	N 34°52'53.69" / E 127°30'55.98"	9	1	3	4	4	5
		5	N 34°52'55.63" / E 127°30'52.68"	22	1	3	6	8	11
		6	N 34°52'59.46" / E 127°30'52.38"	6	1	2	3	4	4
	Tidal flat	7	N 34°52'38.90" / E 127°30'47.23"	9	1	3	5	6	6
		8	N 34°52'34.81" / E 127°30'27.08"	13	1	2	4	9	9
		9	N 34°52'27.65" / E 127°30'14.77"	11	1	3	4	5	6
		10	N 34°52'27.46" / E 127°29'55.06"	16	1	3	5	7	10
		11	N 34°52'27.51" / E 127°29'46.53"	8	1	3	4	5	6
<i>Suaeda japonica</i>	Tidal flat	1	N 34°51'45.94" / E 127°31'10.12"	15	2	5	7	8	10
		2	N 34°51'46.41" / E 127°31'09.65"	18	1	3	7	8	11
		3	N 34°51'49.84" / E 127°31'09.49"	31	2	5	8	12	15
		4	N 34°51'56.41" / E 127°31'06.25"	29	2	5	8	10	13
		5	N 34°51'59.83" / E 127°31'06.25"	23	2	5	8	11	15
		6	N 34°51'59.73" / E 127°31'06.36"	28	1	3	6	7	11

### 2.3. Isolation of Endophytic Fungi

This study adopted culture-dependent methods, in accordance with previous endophyte studies in coastal terrain [15,16] or other environments [17–20]. The samples were quickly transported at 4 °C to the laboratory. Sterile distilled water and sterilized 0.1% Tween-80 solution (Sigma-Aldrich, St. Louis, MO, USA) were sprayed on to the surface of the samples to eliminate suspended solids or normal microflora from the root surfaces. The plants were submerged in 1.0% perchloric acid for 10 min twice and were subsequently washed with sterile distilled water 3–4 times [21]. Residual water was eliminated using dry sterile gauze and 50 pieces of root from one plant sample were cut to a length of 3–4 cm. The pretreated samples were cultured in Hagem minimal medium containing 80 mg/L streptomycin (Sigma-Aldrich) to exclude root bacteria and incubated at 25 °C for 15 days [22,23]. Endophytic fungi were sub-cultured in the same media and under the same conditions. Finally, pure fungi isolates were incubated on potato dextrose agar (Difco, Detroit, MI, USA) and selected based on their morphological differences (Table 1).

### 2.4. Extraction of Genomic DNA and Target Gene Amplification

All of the 324 endophytic fungi collected from the 17 colonies of the two halophyte species native to the three salt marsh regions in Suncheon Bay were inoculated into potato dextrose broth (Difco) and incubated at 25 °C for 7 days at 120 rpm using a rotary shaker. Filtered mycobionts were lyophilized for 2 days. The DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) was used to extract the genomic DNA from the lyophilized mycobionts and primers targeting the universal sequences internal transcribed spacer (*ITS*) 1, partial sequence; *5.8S* ribosomal RNA and *ITS2*, complete sequence; and *28S* ribosomal RNA, partial sequence), *ITS1* (5′–TCC GTA GGT GAA CCT GCG G–3′), and *ITS4* (5′–TCC TCC GCT TAT TGATAT GC–3′) were used for amplification [24]. The polymerase chain reaction (PCR) amplification conditions were as follows: pre-denaturation (94 °C, 4 min), denaturation (94 °C, 1 min), annealing (55–58 °C, 1 min), and extension (72 °C, 2 min) for a total of 35 cycles, followed by a final extension (72 °C, 2 min) [24]. The PCR products were confirmed by gel electrophoresis (1.5% agarose gel, stained with ethidium bromide), and the resulting band pattern was observed under a UV transilluminator. The AccuPrep PCR and Gel Extraction Kit (Bioneer, Daejeon, Korea) was used to purify the PCR products, and the ABI 3730XL DNA analyzer (Applied Biosystems, Waltham, MA, USA) was used for ITS sequencing.

### 2.5. Identification and Phylogeny

A total of 324 fungal isolates were partially identified based on signature sequence homology with data collected from the GenBank database [25]. The sequences of *ITS1* and *28S* (partial sequences), and *5.8S* and *ITS2* (complete sequences) obtained from the fungal isolates were compared with the data deposited in the GenBank database using the BLASTn tool to determine their taxonomical relationships. Furthermore, evolutionary analyses were conducted using Molecular Evolutionary Genetics Analysis (MEGA) version X (64-bit for Windows) [26,27] with sequence alignments prepared using Clustal W version 2.0.10 [28,29]. The evolutionary history was inferred using the minimum evolution method to assess the evolutionary relationships between taxa [30], with ‘associated’ being taxa clustered together in a percentage of the replicate trees in the bootstrap test (1000 replicates) [31]. Evolutionary distances were computed using the Tajima–Nei method [32] and expressed according to the number of base substitutions per site. The minimum evolution tree was explored using the close-neighbor interchange algorithm [33] at a search level of 1. The neighbor-joining algorithm [34] was used to generate the initial tree. All ambiguous positions were removed for each sequence pair (pairwise deletion option). The 324 fungal signature sequences obtained in this study were deposited in GenBank with assigned accession numbers (Supplementary Tables S1 and S2).

### 2.6. Biodiversity Assessment

The diversity of the fungal flora was compared between each halophyte species (Table 2) [35] and the diversity at the genus level was determined using Margalef’s richness [35–37], Menhinick’s diversity [38,39], Shannon’s diversity [40], and Simpson’s indices (1-D) [37,41].

**Table 2.** Diversity of the endophytes from each dominant species flourishing at sampling each site.

Host Species	Sampling Site Character	Site Number	Number of Isolates	Genera Richness	Margalef’s Richness	Menhinick’s Index (Dmn)	Shannon’s Index (H’)	Simpson’s Index (D)
<i>Phragmites australis</i>	Delta	1	25	18	5.281	3.600	2.080	0.027
		2	25	16	4.660	1.919	1.919	0.037
		3	36	16	4.186	2.667	1.434	0.081
		4	9	5	1.820	1.667	1.971	0.139
		5	22	11	3.235	2.345	1.940	0.082
		6	6	4	1.674	1.633	1.663	0.133
	Tidal flat	7	9	6	2.276	2.000	1.739	0.111
		8	13	9	3.119	2.496	1.623	0.064
		9	11	6	2.085	1.809	1.838	0.145
		10	6	10	3.246	2.500	1.595	0.083
		11	8	6	2.404	2.121	1.507	0.107
<i>Suaeda japonica</i>	Tidal flat	12	15	10	3.323	2.582	2.011	0.086
		13	18	11	3.460	2.593	1.572	0.118
		14	31	15	4.077	2.694	1.293	0.075
		15	29	13	3.564	2.414	1.724	0.076
		16	23	15	4.465	3.128	1.502	0.047
		17	28	11	3.001	2.079	1.458	0.106

### 3. Results and Discussion

#### 3.1. Taxonomy and Phylogeny

Among the *P. australis* samples, three phyla (Ascomycota ( $n = 178$ ), Basidiomycota ( $n = 1$ ), and Zygomycota ( $n = 1$ )), seven classes, 13 orders, 25 families, and 41 genera were identified (Table 2). Within *S. japonica*, two phyla (Ascomycota ( $n = 137$ ) and Basidiomycota ( $n = 7$ )), six classes, 12 orders, 20 families, and 35 genera were identified (Table 2). The phylogeny of the endophyte clusters from each site was deduced (Figure S1), showing the phylogenetic relationship between the collected isolates. In total, 58 endophytic genera were secured as a national culture collection in the two species of emerged vegetation from the Ramsar wetlands of Suncheon Bay. All cultures were safely deposited in the National Institute of Biological Resources of Korea. According to the plant taxonomy system, *P. australis* belongs to the kingdom Viridiplantae, phylum Streptophyta, class Magnoliopsida, order Poales, family Poaceae, and genus *Phragmites*. *P. australis* is a herbaceous, perennial, monocotyledonous plant. *S. japonica* belongs to the kingdom Viridiplantae, phylum Streptophyta, class Magnoliopsida, Caryophyllales, family Chenopodiaceae, and genus *Suaeda*, and is a herbaceous, annual, dicotyledon plant [42].

#### 3.2. Endophytic Distribution in Each Emerged Halophyte Species

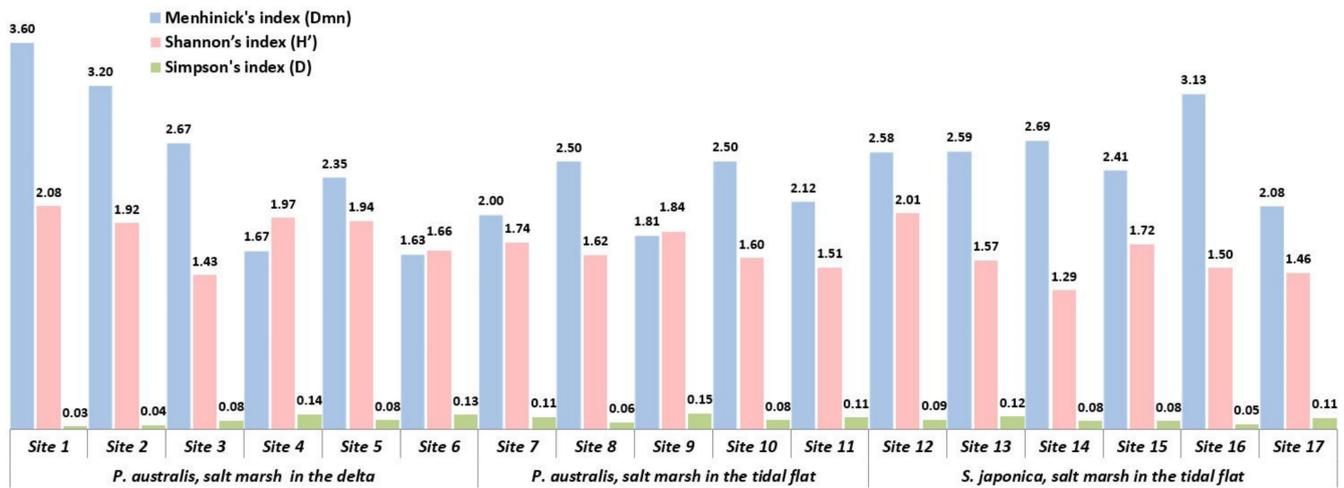
Characteristically, the fungal phylum Ascomycota was highly dominant in the two emerged halophyte species; however, Basidiomycota and Zygomycota, which were widely and densely distributed in the surrounding environment, showed very low ratios. No significant dominance or distribution changes between plant species were observed at the class level; however, a more characteristic distribution was observed at the order, family, and genus levels. Depending on the fungal classification system, the class distribution was Dothideomycetes (dominance: 46.53%) > Sordariomycetes (dominance: 33.33%) > Eurotiomycetes (dominance: 13.19%) in the halophyte species *S. japonica* (Tables S1 and S2). Similarly, dominance and distributions were identified as Dothideomycetes (dominance: 43.33%) > Sordariomycetes (dominance: 38.89%) > Eurotiomycetes (dominance: 13.89%) in *P. australis* (Tables S1 and S2). However, dominance patterns began to show slightly changes when each halophyte species was compared at the order level: with Hypocreales (domi-

nance: 28.89%) > Pleosporales (dominance: 27.22%) > Capnodiales (dominance: 16.11%) in *P. australis* (Tables S1 and S2); and Pleosporales (dominance: 31.25%) > Hypocreales (dominance: 22.92%) > Eurotiales (dominance: 13.19%) in *S. japonica*. At the family level, dominance was the following: Nectriaceae (26.67%) > Davidiellaceae (15.56%) > Trichocomaceae (13.89%) in *P. australis* (Tables S1 and S2); and Nectriaceae (22.92%) > Pleosporaceae (16.67%) > Trichocomaceae (13.19%) in *S. japonica* (Tables S1 and S2). Moreover, several genera commonly showed high dominance, but the ratio and dominance patterns were different: *Cladosporium* (dominance: 15.56%) > *Fusarium* (dominance: 13.33%) > *Penicillium* (dominance: 11.11%) in *P. australis*; and *Penicillium* (dominance: 13.89%) > *Aspergillus* (dominance: 11.81%) > *Cladosporium* (dominance: 11.11%) in *S. japonica*.

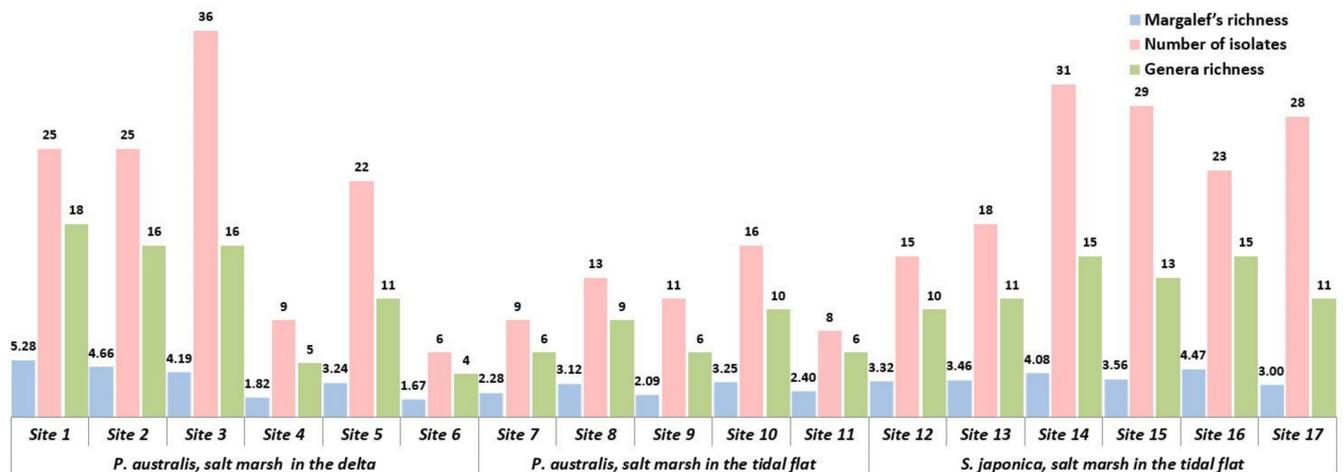
### 3.3. Morphological and Alpha Diversity

There are several purposes for securing diversity information. There are always different forms of geographical and ecological tensions in these various transitional zones, and comparative analysis studies on how some of these tensions have a discriminatory effect on the dominant halophyte and their endophytic clusters are scarce.

Identifying how the ecological tension of the two different ecotones affects the diversity of endophytic fungi sheds light on this purely scientific question. Furthermore, regarding application, information on the diversity of healthy salt marshes can be an indicator for restoring rapidly disappearing coastal wetlands. In addition, the two aquatic plants evaluated herein play an important role as natural purifiers and structural supporters of the aquatic terrain in coastal and freshwater environments, and information on endophytic diversity has clear industrial value. Margalef's, Menhinick's, Shannon's, and Simpson's indices varied within *P. australis* from the delta salt marsh (5.281–1.674, 3.600–1.633, 2.080–1.434, and 0.139–0.027, respectively) and the tidal flat salt marsh (3.246–2.085, 2.500–1.809, 1.838–1.507, and 0.145–0.064, respectively), as well as within *S. japonica* from the tidal flat salt marsh (4.465–3.001, 3.128–2.079, 2.011–1.293, and 0.118–0.047, respectively) (Table 2). In particular, a *P. australis* colony from the delta showed a wide range of genera richness (abundance of genera) of 5.281–1.820 (gap between max. and min.: 3.461), whereas a colony from the tidal flat salt marsh showed a narrower range of 2.276–3.246 (max. and min gap: 0.970). Similarly, the genera richness ranges for *S. japonica* flourishing in the tidal flat salt marsh also showed relatively low gaps (3.001–4.456 (max. and min gap: 1.455)), with a distribution similar to that of *P. australis* in tidal flats. In general, the morphological diversity of the isolates and Shannon's index, genera richness, and Margalef's richness tends to be proportional (Shannon's index encompasses the evasiveness and species diversity of a particular cluster). In particular, sites 4 and 6, which had low Shannon's index or morphological diversity (Table 2), also showed low levels of richness and dominance of certain genera. Menhinick's richness and Shannon's indices were roughly proportional among the samples (Figure 3A), with the exceptions of sites 4 and 6, which are inland areas of the delta (Figure 2), owing to their low degree of morphological diversity (i.e., low number of isolates due to morphological differences); in addition, certain dominances were also low. Furthermore, the Simpson's index, which reflects the dominance of a specific species, tended to be inversely proportional to the other indices (Figure 3A). Morphological diversity and genera richness were generally proportional (Figure 3B), with exception of the values at sampling site 16, as the endophytes collected from this site included several genera with higher dominances. In the case of site 15, for *S. japonica* in the tidal flat salt marsh, there was low genera richness; however, the existence of certain genera of *Penicillium* with higher dominance resulted in the observed inversely proportional values between Menhinick's index and Shannon's diversity.



(A)



(B)

**Figure 3.** (A) Variation of Menhinick's, Shannon's, and Simpson's indices at each sampling site. (B) Variation of morphological diversity and richness (genera and Margalef's index) at each sampling site.

### 3.4. Endophytic Distribution and Diversity within Salt Marshes in Estuaries and Tidal Flats

Even with colonies of the same plant species, the pattern of variation in the distribution and diversity of endophytes differed depending on the type of the transitional zone (Table S3). In the case of *P. australis* colonies that flourished in the delta salt marsh of a river estuary, 3 fungal phyla (namely Ascomycota, Basidiomycota, and Zygomycota), 6 classes, 10 orders, 18 families, and 34 fungal genera were identified, whereas in the *P. australis* colonies that flourished in the tidal flat salt marsh, only 1 phylum (namely Ascomycota), 4 classes, 6 orders, 11 families, and 19 genera were identified (Tables S1 and S2). Morphological fungal diversity in the wetlands in the tidal flat salt marshes was estimated to be less diverse than that in the wetlands formed in the delta of the estuary, which was clearly revealed via diversity analysis.

For *P. australis* native to the six sites that flourished in the delta salt marsh in the river estuary, four sites showed higher diversity compared with the *P. australis* colonies from the tidal flat salt marsh. Morphological diversity and the change patterns of genera richness, Margalef's richness, and Shannon's index were almost identical (Figure 3A). The algebraic diversity of *P. australis* colonies formed at the delta of the river estuary was also much higher than that of those formed at the tidal flat salt marsh (Figure 3A). However, some sites in the delta showed very low endophytic diversity, which widens the gap in diversity indices between the sampling sites in the delta salt marsh. It is reasonable to

assume that sites 4 and 6 suffer differentiated ecological selective pressures caused by harsh environments. Moreover, they showed the highest Simpson's index among all survey points (0.139 and 0.133, respectively; Figure 3A); therefore, the strong selective pressure may have affected the dominance of certain fungi since the area surveyed has a dense *P. australis* colony and the distance between the sites is more than 100 m, making ecological exchange between colonies difficult. Hence, it is possible that the widened gap of the biodiversity values between each site in the delta salt marsh in the estuary compared with those between the sites in the tidal flat could be due to the fact that the river estuary is a different type of ecotone (transitional zone), with extreme environmental conditions changes and thus more diverse ecological tension imposed on the colonies.

In the case of tidal flats, the key characteristic of the transitional zone is simply the space where the ground meets sea level. The stress that vegetation must endure is a marine environmental factor, such as tide ranges in a constant cycle or salt accumulation [43]. In contrast, the most important variable characteristics of river estuary water are the concentration of dissolved oxygen, salinity, and sediment load. There is extreme spatial variability in salinity, with a range of near zero at the tidal limit of tributary rivers up to 3.4‰ in the delta. Hence, salinity will vary considerably over time and through the seasons at each point, making it a harsh environment for organisms. Sediment often settles in flats, which are extremely difficult to colonize. No points of attachment exist for algae; therefore, a vegetation-based habitat cannot be established. Sediments can also clog the feeding and respiratory structures of species, and special adaptations exist within mud flat species to cope with this problem. Lastly, the variation in dissolved oxygen can cause problems for life forms. Nutrient-rich sediment from man-made sources can promote primary production life cycles, possibly leading to eventual decay removing the dissolved oxygen from the water; therefore, hypoxic or anoxic zones may develop. Thus, river estuaries are incredibly dynamic systems where temperature, salinity, turbidity, depth, and flow change daily in response to the tides. This dynamic environment makes estuaries highly productive habitats, but also make them difficult for many species to survive in [43].

Therefore, characteristics of the delta established in the river estuary are more diverse than those of the general tidal flats. It is an area where the ground meets sea level, and where freshwater and seawater meet. In addition, there is a flow of water due to tidal ranges; however, the flow rate is not constant, depending on the weather and climate changes, and sometimes depending on flooding. Therefore, the concentrations of salt and organic/inorganic matter are not always constant. This drastic change will widen the gap in environmental conditions between sampling points.

In contrast, relatively fewer dynamic variations were observed in the endophytic diversity in *P. australis* or *S. japonica* colonies formed in the marshes of tidal flats, which is estimated to be due to the constant ecological selective pressure originating from the tidal range. This is not a place where the massive freshwater flows of large rivers occur; it only experiences tidal ranges or consequent salt intrusion. From these results, it can be inferred that delta marshes formed in large river estuaries are much more exposed to environmental (aquatic geography) stress than marshes formed in tidal flats, and that the endophytic clusters of the dominant emerged halophyte communities flourishing in deltas are also greatly affected. *P. australis* colonies and their endophytes, which thrive in overcoming these dynamic environmental changes, may provide important clues to the future restoration of the salt marshes that are currently disappearing. Once the vegetation in coastal sand dunes or salt wetlands becomes damaged, the environment changes at a rapid pace as it is exposed to the strong stress introduced by the impaired protection provided by the damaged coastal vegetation.

In some of the few studies available on endophyte microorganisms of wetlands located in the coastal ecotone, bacterial communities are reported to possess variable characteristics depending on the salt concentration in the super-tidal zone [44]. In particular, the establishment of *P. australis* as a symbiotic halophyte plant reported in a previous study was also observed in the present study. In addition, herein, we found that the

endophytic fungal diversity distinctively contrasted between the delta and the tidal flat areas. Furthermore, from the perspective of securing biological resources, a variety of novel species of endophytic microorganisms were reported in halophytes that are thriving in coastal ecotones [45–48]. This may represent the existence of a new microbial resource that has evolved and adapted to the particular selective pressures in the coastal ecotone zone, where changes are always extreme. In addition, considering the unique diversity variation of endophytic fungi in the coastal ecotone, further investigations on and culture collections of the microbial diversity of these unique coastal areas are warranted. Of note, one purpose of securing a culture collection of unique environments is to secure the new genetic resources of novel microorganisms [9]; in addition, nowadays, novel strains are also related to the competitiveness of the value of biological resources [10,49].

### 3.5. Wide Distribution of Certain Fungal Genera

Unexpectedly, despite the ecological tension and selective pressures at the river estuary, some sites may have more diverse genera than the tidal flats, which have relatively stable terrain conditions. This means that despite the short intervals (100 m), changes in terrain condition are dramatic, which also significantly affect the distribution of endophytic fungi in ecologically isolated native halophyte colonies. However, there were commonly eight genera distributed among the three regions that overcame such dramatic variations in terrain conditions, namely *Aspergillus*, *Cladosporium*, *Epicoccum*, *Fusarium*, *Paraconiothyrium*, *Penicillium*, *Septoriella*, and *Talaromyces*. In the case of *Aspergillus* and *Cladosporium*, the dominance ratio in each site (vegetation) was very high, whereas other genera, namely *Epicoccum*, *Paraconiothyrium*, *Septoriella*, *Talaromyces*, did not show dominance, being widely distributed in host vegetation regardless of the site. Previous reports described *Epicoccum* as a marine terrain-distributing fungus [50] and *Talaromyces* as a transitional zone fungus [51]. *Aspergillus*, *Penicillium*, and *Talaromyces* were reported as marine terrain-distributing fungi, with higher functions or a rock solubilizing function in marine terrains [52–57]. In the present study, 11 fungal genera were found to be commonly shared within the two tidal flats regardless of the host species (26.8% of the total genera secured in this study), namely *Alternaria*, *Aspergillus*, *Cladosporium*, *Epicoccum*, *Eserohilum*, *Fusarium*, *Paraconiothyrium*, *Penicillium*, *Phialemoniopsis*, *Septoriella*, and *Talaromyces*. Recently, *Eserohilum* was reported as an endophytic fungus [58,59]. Herein, most of these fungi were identified as endophytes in the salt marshes that developed in tidal flats and the river estuary. Taken together, *Aspergillus*, *Cladosporium*, *Epicoccum*, *Paraconiothyrium*, *Penicillium*, *Septoriella*, and *Talaromyces* are endogenized in a wide range regardless of the ecotone condition. In particular, *Aspergillus*, *Cladosporium*, and *Penicillium* are generally dominant in wide vegetation, and because the secured culture collection within these genera has morphological diversity, it is predicted that there will be a variety of species within them. In other words, the common characteristics of these genera are believed to help endogenize the species within them, thereby overcoming environmental stress and dramatic variation in terrain conditions derived from a specific ecotone, such as a transitional zone with ecological tension.

Further, this phenomenon provides another useful clue for culture collection strategy. Figure S2 shows the taxonomic information of endophytic fungi that are commonly distributed, thereby overcoming host plant species variation and diverse ecotone environments. Hence, these fungal species are likely to be resistant to the extreme, rapidly changing environment, holding potential as culture collections for the future restoration of destroyed coastal wetlands.

## 4. Conclusions

This is the first study to compare and analyze how different ecological tensions affect the diversity and distribution of endophytes by comparing delta and tidal flat salt marshes in the Ramsar wetlands of Suncheon Bay on the Korean Peninsula. One purpose of this study was to secure a national culture collection (in addition to the pure ecological

study). In summary, this study demonstrated that the potential symbiotic endophyte fungal community shows wider biological diversity variation in relatively extreme coastal ecotones (more in the delta than in tidal flats). Furthermore, despite the extreme ecological selective pressures, 11 fungal genera found in both deltas and tidal flats were identified. Therefore, these particular taxa that can overcome a variety of ecological pressures may be used in the future restoration of destroyed coastal environments. Additional studies addressing the host halophyte–microbe relationship are needed.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/jmse9040430/s1>, Figure S1: Phylogenetic tree of endophyte fungi; Figure S2: Distribution of fungal endophyte symbiosis with each halophyte species and colony; Table S1: Fungal endophyte isolates from host *Phragmites australis* species clusters; Table S2: Fungal endophyte isolates from host *Suaeda japonica* species clusters; Table S3: Distribution of the endophytic genera from each site for each halophyte species.

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