

New distribution record of seagrass *Ruppia brevipedunculata* from intertidal cultivation ponds in South Kalimantan, Indonesia

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Abstract. *Salim D, Ambo-Rappe R, Mashoreng S, Kadir NN, Zakaria MH, Kiswara W. 2025. New distribution record of seagrass Ruppia brevipedunculata from intertidal cultivation ponds in South Kalimantan, Indonesia. Biodiversitas 26: 2088-2096. Ruppia* seagrass has a widespread distribution across global waters. The loss of *Ruppia* in the water highlights the broader environmental challenges faced by coastal ecosystems due to human activities. This study aimed to investigate the distribution of *Ruppia brevipedunculata* in South Kalimantan Province, Indonesia and identify it using both morphological characteristics and phylogenetic analysis. Samples of *Ruppia* were randomly collected from cultivation ponds using linear transects of 100 meters in length. The samples were then measured to describe their morphological characteristics. For molecular identification, a total of 200 mg of fresh leaves was isolated using the Plant Genomic DNA Mini Kit and then amplified using the ITS gene sequence, followed by the PCR procedure. DNA analysis was conducted using MEGA 11 software, which involved editing, trimming, and aligning sequences with CLUSTAL W, constructing a phylogenetic tree, and calculating genetic distances. The sample of *R. brevipedunculata* and other *Ruppia* sequences were analyzed using the BLAST. The findings revealed that the morphological characteristics of the samples collected in this study closely matched those of *R. brevipedunculata* recorded in other regions. Furthermore, phylogenetic analysis showed that *R. brevipedunculata* from this study is closely related to populations from China and Rote Island, Indonesia. This study contributes valuable data on the newly identified distribution record of *Ruppia* in Indonesian waters and highlights the importance of preserving their habitat in coastal areas.

Keywords: Cultivation ponds, morphological and molecular, preserve, *Ruppia brevipedunculata*, South Kalimantan

INTRODUCTION

Ruppia is a genus from the family of Ruppiales. This seagrass is mainly characterized by short peduncle and small fruits. Their habitat are permanent or temporary shallow waters, ranging in depth from 0.1 to 1 meter (Yu and den Hartog 2014). This genus has been described to contain 13 species, namely as *R. bicarpa*, *R. brevipedunculata*, *R. cirrhosa*, *R. didyma*, *R. drepanensis*, *R. filifolia*, *R. maritima*, *R. megacarpa*, *R. tuberosa*, *R. polycarpa*, *R. sinensis*, *R. mexicana* n. sp., and *R. mongolica* (Yu and den Hartog 2014; den Hartog et al. 2016; Ito et al. 2017; den Hartog and Triest 2020; Saood et al. 2022; Munari et al. 2023; Zou et al. 2023). Among them, 11 species have been accepted in the international taxonomical database of world plants (POWO 2024), while two species (*R. mexicana* n. sp., and *R. mongolica*) are two newly identified species (den Hartog et al. 2016; Zou et al. 2023). Among them, *R. maritima* and *R. cirrhosa* have wide distribution in the waters around the world, while the

other species have limited distribution to particular countries or waters (Zou et al. 2023).

The research on the distribution of *Ruppia* seagrass in Indonesian waters is still scarce, even though this plant plays a crucial role both ecologically and economically. *Ruppia* meadows play an important ecological function in aquatic ecosystems, such as providing critical habitat not only for endangered animals but also for various aquatic animals (invertebrates, small fish, and waterfowl), increasing the diversity of macrobenthos, and improving the nutritional quality, especially the carbohydrate component, of organic matter in sediment (Kantrud 1991; Mannino et al. 2015; Martínez-Garrido et al. 2017; Munari et al. 2023). Due to their high absorbing capacity, this plant has high potential as a bioindicator and biomonitoring of heavy metals and oil spills in coastal waters (Martin et al. 2015; Gu et al. 2021), been used for the assessment of water quality (Martin et al. 2015; Mustafa and Hayder 2021; Edo et al. 2023; Makki et al. 2023), been used as phytoremediation agents to treat wastewater produced from

the industrial, agricultural, and domestic sectors (Mustafa and Hayder 2021; Kafle et al. 2022). In economic context, this plant has been used in cosmetics, foods, and pharmaceutical industries (Chaabani et al. 2023). Extracts from the *Ruppia* plant contain bioactive compounds which act as antioxidants and antimicrobials, such as phenol, tannin, chicoric acid and flavonoid glycosides (Enerstvedt et al. 2017; Chaabani et al. 2023). This plant is also reported to be a source of *ent*-labdanes diterpenes, which are compounds known as antialgal (*Selenastrum capricornutum*) (Dellagreca et al. 2000; RochaDutraNobre 2024). In animal husbandry, this plant is used as an alternative feed source for fattening sheep (Saragih et al. 2024). Therefore, as research on *Ruppia* seagrass continues to advance rapidly, and given the lack of sufficient data on this plant in Indonesian waters, it is essential to conduct thorough and adequate research.

Previous studies have documented the occurrence of *Ruppia* solely from the species *R. maritima* in the waters near Indonesia, including Malaysia, Australia, Philippines and Vietnam (Bujang and Zakaria 2003; Coles et al. 2003; Fortes 2003). However, in Indonesian waters, this plant has only been recorded in herbarium collections at the Herbarium Bogoriense, the specimen herbarium was made in 1847 by Zollinger and Kostermans in 1962 from Ancol, Jakarta Bay and Pasir Putih, East Java, Indonesia (Kuriandewa et al. 2003). The majority of the research on *Ruppia* mentioned above focused solely on the description of *R. maritima*, which raises the possibility that this species could actually be *R. brevipedunculata*. This assumption is based on the fact that *R. maritima* has only been described in Africa, Europe, and the Mediterranean (Kurniawan et al. 2024). This gap underscores the necessity for further studies to explore the presence of *R. brevipedunculata* in Indonesian waters. Recently, *R. brevipedunculata* has been rediscovered for the first time on Danau Laut Mati or Dead Sea Lake in Rote Island, East Nusa Tenggara, Indonesia (Kurniawan et al. 2024). This species was previously identified as *R. maritima* (Yu et al. 2014) but was reclassified by Yu and den Hartog (2014), who reinstated it as *R. brevipedunculata*. Based on this, *R. maritima* found in Indo-Pacific waters represents a distinct clade and should be classified as *R. brevipedunculata* (Kurniawan et al. 2024). Therefore, this study will also expand the distribution database of *R. brevipedunculata* established by Kurniawan et al. (2024), who only identified it in high salinity lake waters.

The current understanding of the distribution of *R. brevipedunculata* in Indonesia remains limited. To date, Rote Island is among the few locations where the presence of this species has been formally documented. Comprehensive data on its occurrence in other regions of the country are lacking. Nevertheless, given its ecological preference for shallow, brackish to saline aquatic environments, it is plausible that *R. brevipedunculata* may inhabit other similar coastal habitats throughout the Indonesian archipelago. Further field investigations and species-level identification are necessary to confirm its broader distribution. South Kalimantan was selected as the focal area for this study based on several key

considerations. First, there were preliminary reports indicating that *Ruppia* species are known to tolerate a wide range of salinities, from near-freshwater to hypersaline environments (Strazisar et al. 2014; Yu and den Hartog 2014). Second, the area encompasses a variety of habitats that align with the ecological preferences of *R. brevipedunculata*, including shallow brackish lakes, seasonal wetlands, and river mouths, all of which may provide suitable environmental conditions for the species. Third, compared to other regions such as Java and eastern Indonesia, South Kalimantan remains relatively understudied in terms of seagrass and submerged aquatic vegetation diversity. This presents a valuable opportunity to generate novel data and contribute to the broader understanding of aquatic plant distribution in Indonesia. Taken together, these factors highlight both the ecological relevance and the research potential of South Kalimantan as a study site.

This study aims to explore the newly identified geographical distribution of *R. brevipedunculata* in South Kalimantan, Indonesia focusing on its morphological characteristics and molecular properties. This research is essential not only for confirming the presence of this elusive species but also for validating its taxonomic classification. Moreover, the findings will lay the groundwork for developing future conservation strategies aimed at protecting and restoring *Ruppia* populations in Indonesia's coastal ecosystems, ensuring their long-term sustainability.

MATERIALS AND METHODS

Study area and period

This study was conducted on December 25, 2023 in South Kalimantan Province, Indonesia, with sampling was carried out at two cultivation ponds located at the geographical coordinates of 3°28'47.33" S, 116°00'23.91" E, and 3°30'51.23" S, 115°59'30.05" E (Figure 1). Administratively, the sampling sites were in the Kersik Putih and Segumbang Villages, Batulicin Sub-district, South Kalimantan Province.

Procedures

Sample collection

A total of ten seagrass specimens were randomly sampled from the two ponds using a 100-meter transect line (Martínez-Garrido et al. 2017). At each pond, environmental parameters were measured including water temperature, salinity, and water depth. The samples of leaves, stems, rhizomes, fruits, and flowers were collected by hand-pulling and placed in a Ziplock bag containing silica gel and then brought to laboratory for further analysis. In the laboratory, seagrass samples were washed several times with pure water to remove attached algae and mud, then stored in a clean, airtight plastic container and placed in a refrigerator at a temperature of -18°C (Saood et al. 2022). These wet specimens were preserved for Deoxyribonucleic Acid (DNA) analysis.

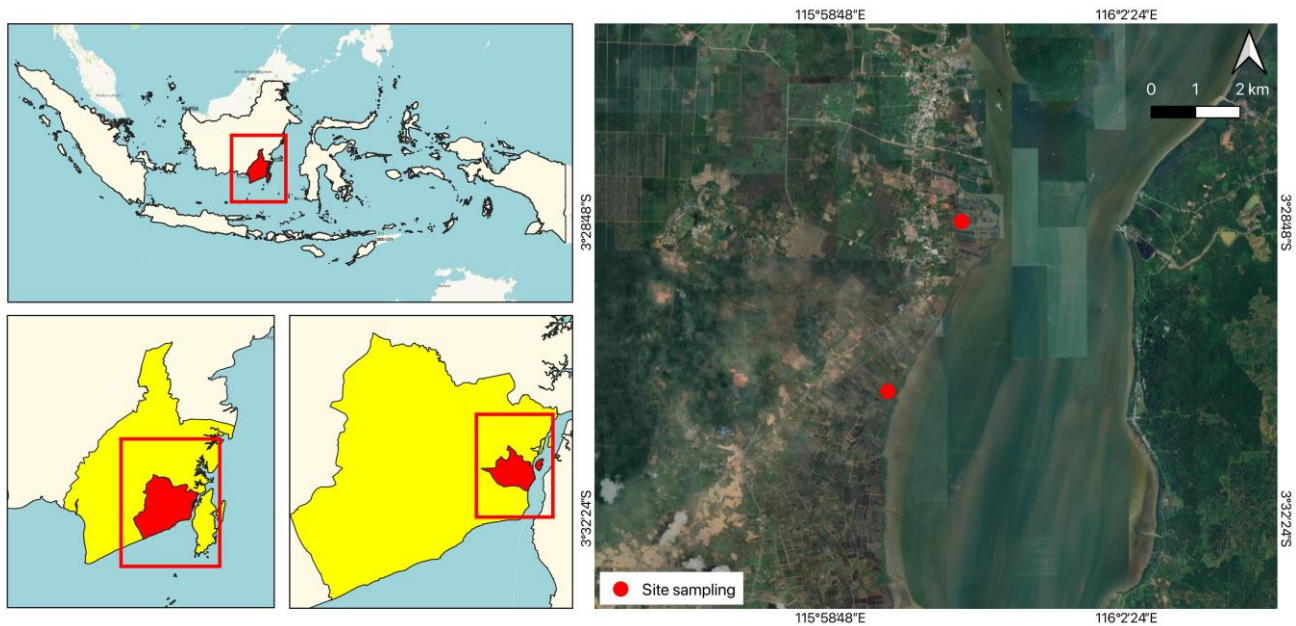


Figure 1. Map of the sampling locations in the Kersik Putih and Segumbang Villages, Batulicin Sub-district, Tanah Bumbu District, South Kalimantan Province, Indonesia

For herbarium specimens, after collection, the samples were placed between sheets of newspaper or blotting paper and then pressed to flatten it. The pressing process may take several days to a week to properly dry and preserve the plant materials. Once the plant was fully dried, it was mounted onto a standard-sized herbarium sheet. The specimen was attached using glue, tape, or pins. It was placed in a way that shows key identification features, such as leaves, fruits, flowers, and stems. Herbarium specimen was labeled with important information (scientific name, date and location of collection, and collector's name). Once mounted and labeled, specimens were stored in the herbarium cabinets at the Genetic and Molecular Biology Laboratory, Universitas Lambung Mangkurat, South Kalimantan. Herbarium specimens were archived in a way that allows easy access for study.

Morphological identification

The photographs and morphological data of the seagrass were taken using Olympus Tough TG-6 camera and Biological Microscope Model Number B340 & T340 and reviewed according to their morphological characteristics (Yu and den Hartog 2014; Mannino et al. 2015; Ito et al. 2017; Martínez-Garrido et al. 2017). The morphological description of *Ruppia* plants was based on the measuring of minimum, maximum and average of each specimen's morphological characteristics (Martínez-Garrido et al. 2017). This included 10 characters for plant height; 40 for internode length, leaf length and leaf width; 39 for root length and rhizome diameter; 36 for inflorescence peduncle length and diameter, fruit length, fruit width, podogynes length and number of ripe fruits per flower; 16 for the length and width of seeds, and 8 for carpel number (Martínez-Garrido et al. 2017; Zou et al. 2023). The morphological characteristics of each specimen

were measured using a Digital Slide Caliper (CD-6"-CSX Code 500-196-20, Mitutoyo Digimatic Absolute, Japan) and a stainless-steel ruler.

Molecular analysis

The genomic DNA of *R. brevipedunculata* was extracted from 200 mg of fresh leaves according to the methodology provided by the manufacturer of the DNA Extraction Mini Kit (Geneaid, New Taipei City, Taiwan). The isolated DNA sequence was then amplified using the ITS gene (forward and reverse). All amplification reactions were carried out in a Polymerase Chain Reaction (PCR) thermocycler (Multigene Optimax Labnet). PCR products (amplicon) were separated and visualized on a 1.5% agarose gel. The amplified DNA fragments, according to the expected size were then collected from the amplicon and analyzed (sequencing) using the Sanger method (Saood et al. 2022).

For bioinformatics analysis, the entire sequence was accessed from the GenBank database or the National Center for Biotechnology Information (NCBI). After that, homology analysis (similarity) to other *R. brevipedunculata* species was performed using the BLAST software (also available on the GenBank page). GenBank has a comprehensive database of nucleotide sequences or gene descriptions that can be accessed freely (Sayers et al. 2019). Genetic relationship analysis of *R. brevipedunculata* species was performed using MEGA 11 software by entering and changing the data of each sequence, from notepad format to Mega (.meg) format (Tamura et al. 2021). Finally, statistical analysis of the internal branches of the formed phylogenetic tree was performed using the *Neighbor-Joining* bootstrap 1000 times method with Kimura 2-parameter model (Kumar et al. 2018).

RESULTS AND DISCUSSION

Morphological characteristics

Ruppia brevipedunculata found in the intertidal zone in cultivation ponds of South Kalimantan are characterized by several morphological characteristics. The morphological characteristics of vegetative and reproductive parts of *R. brevipedunculata* is presented in Figure 2 and Table 1. The samples of *R. brevipedunculata* were collected in the cultivation ponds in Kersik Putih and Segumbang Villages, Batulicin Sub-district as shown in Figure 2.A. This species is submerged with robust stature and branches (Figure 2.B). It has a height ranging from 20.9-69.5 cm with average 41 cm. The rhizome spreads with a diameter of 0.2-0.7 with average 0.3 mm. From each internode segment, one root grows downwards with an internode length of 0.6-2.7 cm with average 1.6 cm and a root length of 1.6-10 cm with average 5.3 cm.

The leaves are straight with a sharp tip (Figure 2.C) with a length of 2.5-11.9 cm with average 6.6 cm and a width of 0.2-0.4 mm with average 0.3 mm. They have a distinct midrib with light green color and contain numerous tannin

cells. The midrib ends just before the acute leaf tip, and the leaf margin near the apex has several small teeth. The leaf sheath has two ear leaves at the top and flower clusters surrounded by a leaf sheath (Figure 2.D). The short inflorescence peduncle (curve shape) emerges from the leaf sheath with a diameter of 0.2-0.6 mm with average 0.3 mm and a length of 3-9.5 mm with average 5.9 mm. Fruits are asymmetrical, ovate, and brown color (Figure 2.E) with a podogyne length of 0.3-1.8 cm with average 1.1 cm. Mature fruits with a beak about 1.2-2.9 mm with average 2.1 mm in length and a width of 0.8-1.6 mm with average 1.2 mm. The gynoecium is composed of 2-5 carpels per flower (Figure 2.F). The free carpels, with no style. The ovary is bottle-shaped and has a fleshy, peltate stigma, containing a single ovule. Each flower with one short inflorescence peduncle has two to four ripe fruits (Figure 2.G). The fruits are easily detached and soon become seeds. Once the exocarp decays, the endocarp is black with a beak about 0.1-0.9 mm with average 0.4 mm long and curving at the tip. The seeds about 1.3-2.7 mm with average 1.9 mm in length and a width of 0.5-1.5 mm with average 1 mm (Figure 2.H).

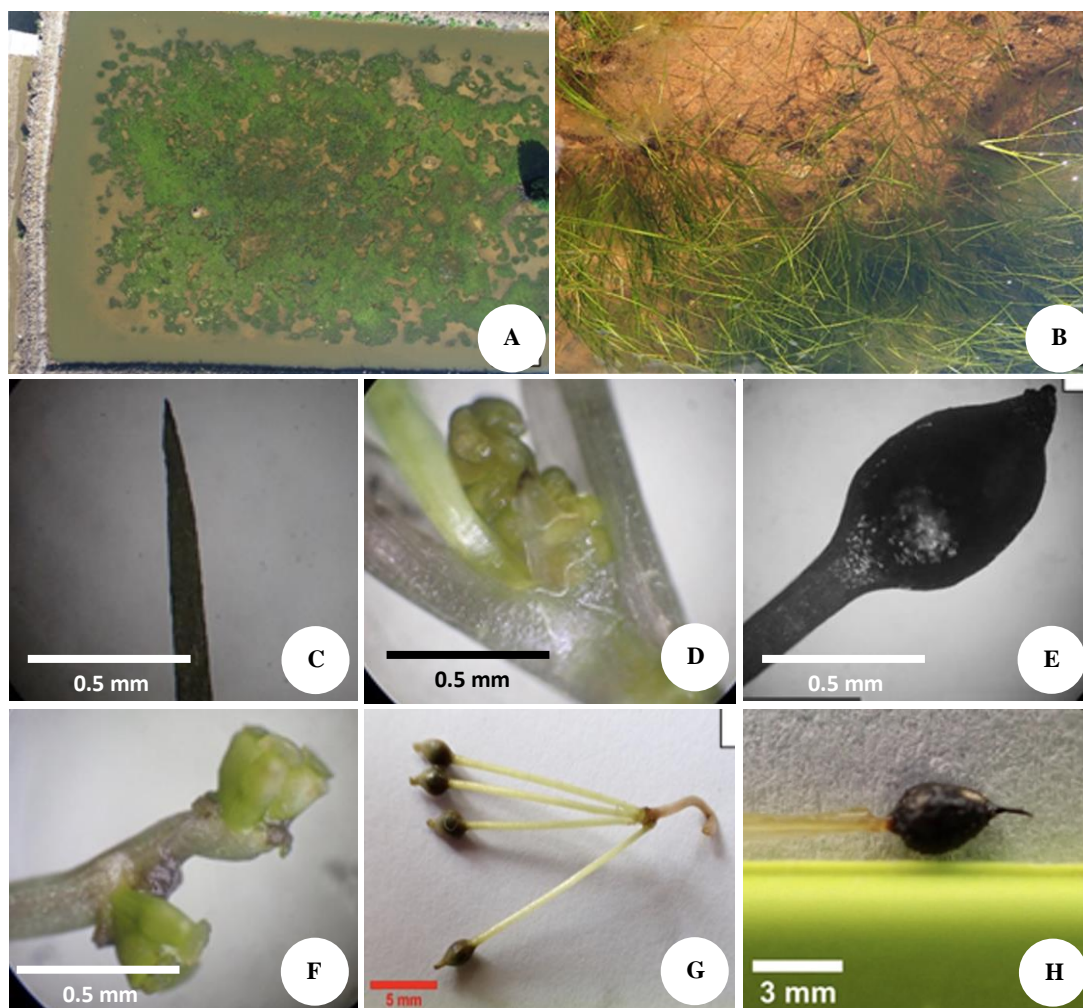


Figure 2. Morphological appearance of *Ruppia brevipedunculata* in South Kalimantan, Indonesia. A. *R. brevipedunculata* in the cultivation ponds; B. The submerged plants; C. The pointed leaf tip; D. The flower cluster; E. The ripe fruit; F. The carpel per flower; G. The podogyne and four ripe fruits; H. The seed with a beak

Table 1. Measurements of morphological characteristics of *Ruppia brevipedunculata* plant based on minimum, maximum and mean±SD

Characteristic features, (no. of sample)	Minimum	Maximum	Average±SD
Vegetative			
Plant height (cm), (n: 10)	20.9	69.5	41±13.02
Internode length (cm), (n: 40)	0.6	2.7	1.6±0.4
Leaf length (cm), (n: 40)	2.5	11.9	6.6±1.6
Leaf width (mm), (n: 40)	0.2	0.4	0.3±0.05
Leaf sheath length (cm), (n: 40)	0.4	1.6	0.9±0.2
Rhizome diameter (mm), (n: 39)	0.2	0.7	0.3±0.1
Root length (cm), (n: 39)	1.6	10.0	5.3±1.4
Reproductive			
Fruit beak length (mm), (n: 36)	0.1	0.9	0.4±0.2
Podogyne length (cm), (n: 36)	0.3	1.8	1.1±0.3
Inflorescence peduncle length (mm), (n: 36)	3.0	9.5	5.9±1.1
Inflorescence peduncle diameter (mm), (n: 36)	0.2	0.6	0.3±0.09
Fruit length (mm), (n: 36)	1.2	2.9	2.1±0.3
Fruit width (mm), (n: 36)	0.8	1.6	1.2±0.2
Carpel length (mm), (n: 8)	0.4	1.1	0.7±0.09
Seed length (mm), (n: 16)	1.3	2.7	1.9±0.2
Seed width (mm), (n: 16)	0.5	1.5	1.1±0.2

Molecular analysis

In general, the results of the reconstruction of the phylogenetic tree of *R. brevipedunculata* using ITS gene produce two clades. The first clade consists of several species including *R. maritima* (Canada and Vanuatu), *R. cirrhosa* (China and Canada), *R. drepanensis* (Italy) and *R. megacarpa* (China). While the second clade consists of two species, *R. brevipedunculata* (China and Rote Island-Indonesia) and *R. maritima* (France and India) (Figure 3). The value on the clade branch is the bootstrap values expressed in percentage form in the *Neighbor-Joining* (NJ) method. In the second clade, the value on the branch is 100% and 81% in sub clade. This value shows the position of the phylogenetic tree which has many similarities in its sequence order. The NJ method is a distance-based method that oversimplifies the evolutionary process, limiting its accuracy, especially for complex or deep evolutionary relationships. In this regard other methods, such as Maximum Likelihood or Bayesian Inference, give more accurate and detailed results. However, the choice of method depends on the specific goals of the analysis, the complexity of the data, and the computational resources available. In this research, the NJ is a useful method for quick, efficient tree-building and relatively accurate for general use (Saitou and Nei 1987).

Discussion

The recent study presents a new distribution seagrass of *R. brevipedunculata* in South Kalimantan, Indonesia. *Ruppia brevipedunculata* in the cultivation ponds is an adaptable plant species that can grow as both a perennial and an annual in the intertidal zone of South Kalimantan. The ponds, which are currently used to cultivate fish and shrimp, used to be mangrove swamps, which have changed a lot since the 1990s due to human activities such as traditional fishing. Since the 1990s, the presence of seagrass in these waters has never been reported. In contrast, *Ruppia* has been reported in cultivation ponds in

other parts of the world, such as Pulandian, Liaoning, and Shandong Provinces in China (Yu and den Hartog 2014; Gu et al. 2021), and in Tam Giang, Vietnam (Triest et al. 2021).

In these ponds, *Ruppia* plants grow abundantly and prefer brackish, shallow, stagnant, transparent waters, soft muddy and clay substrate textures. The salinity and temperature in the ponds ranged from 15.30-28.93 ppt and 28.80-30.60°C, respectively. A comparison of our samples with *R. brevipedunculata* from the Dead Sea Lake on Rote Island revealed significant differences in habitat characteristics. The waters in this region had salinity levels higher than seawater (40-42 ppt) and are minimally influenced by riverine inflows, with little known about any impact from the sea (Kurniawan et al. 2024).

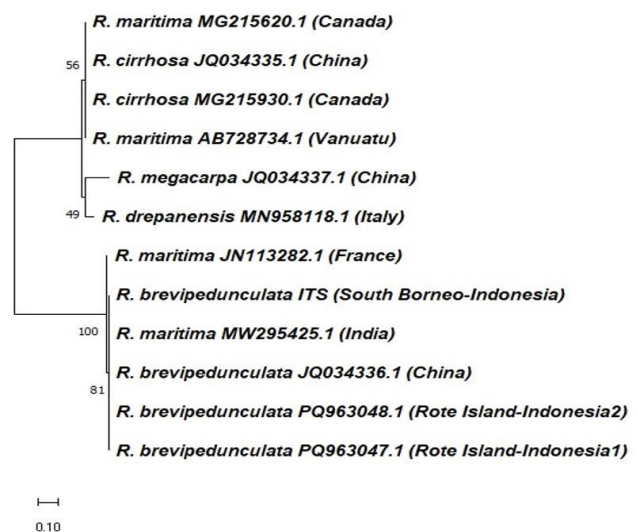


Figure 3. Phylogenetic tree of *Ruppia brevipedunculata* and other species under the *Ruppia* genus based on Internal Transcribed Spacer (ITS) sequence

However, *Ruppia* seagrass can respond to various abiotic factors, including seasonal differences, salinity, temperature, and drought conditions (Strazisar et al. 2021). This species has been found to tolerate salinity and hydrology variations at the ecotones between the freshwater Everglades marshes and Florida Bay, a subtropical estuary with seasonally hypersaline conditions, where salinity can range from 0 to 70 psu. The fluctuating salinity gradient at the ecotone is influenced by factors such as evaporation and precipitation patterns typical of a subtropical climate, upstream freshwater inflows, and wind-driven tides from Florida Bay (Strazisar et al. 2014). The sustainability of seagrass populations in coastal areas largely depends on their ability to adapt to varying hydrological conditions, salinity, and water quality (Jiang et al. 2015; Koch et al. 2015). This adaptability is closely linked to reproductive activity that ensures population maintenance. The primary mechanisms for persistence in dynamic environments include asexual reproduction through clonal growth of vegetative shoots and rhizome fragmentation, as well as the recruitment of new individuals (genets) from seed or propagule banks (Strazisar et al. 2021).

Ruppia meadows in this study are monospecific and appear sporadically, either permanently or temporarily, in ponds with depths ranging from 0.3 to 1 meter, depending on the water level, which is influenced by the actions of fishermen managing the ponds and seasonal changes, such as during the rainy season. It seems that *Ruppia* seeds can survive for several months during droughts in the dry season. For example, one of the ponds in this study was dry for about five months, but after rainfall and with fishermen adjusting the water levels of cultivation, *Ruppia* began to grow well in the following months. It is likely that the seeds were buried in the substrate and survived until the pond was refilled with fresh and marine water. Temporary droughts were also observed when fishermen harvested the ponds, typically drying them for about three days before reintroducing seawater for the next cultivation cycle. During this time, some *Ruppia* plants were still able to

survive. *Ruppia* plants can adapt to temporal abiotic and biotic factors, including seasonal changes and competition with other Submerged Aquatic Vegetation (SAV) (Strazisar et al. 2021).

In these ponds, we also found *Ruppia* growing together with filamentous algae and other SAV (e.g. *Najas marina*). Macroalgae can associate with seagrass plants by living attached or free-floating and forming a dense layer that covers the seagrass plants (Emmclan et al. 2022). The presence of *N. marina* at the study site is still limited (1 or 2 patches) because the inflow and outflow of seawater in these cultivation ponds are still controlled by fishermen. This plant thrives when the rainy season is sufficiently high or the cultivation ponds are flowed with water from large rivers, such as the Barito River in South Kalimantan. Both *Ruppia* and *Najas* in these ponds play crucial roles in food production, primary productivity, and providing habitat diversity for other aquatic organisms (Edo et al. 2023).

Based on the comprehensive morphological description obtained from this study, the species is provisionally identified as *R. brevipedunculata*. In terms of morphological characteristics, *Ruppia* seagrass can be distinguished by looking at leaf width, leaf tip, and the number of carpels (Yu and Hartog 2014). The morphometric values of this study do not show the same size with the Yu and den Hartog (2014) and Kurniawan et al. (2024) but were within the range of their results (Table 2). Thus, the short, brown colored and curve shape of inflorescence peduncle (Figure 2.G) was strongly consistent and similar to *R. brevipedunculata* from China and Rote Island-Indonesia (Yu and den Hartog 2014; Kurniawan et al. 2024). Statistically, the range of morphometric values presented in Table 2 shows similar values across the study sites. In fact, some characteristic features, such as leaf sheath length, podogynes length, inflorescence peduncle, and number of carpels, exhibit identical values. The differences in size for these characteristics may be related to the varying number of sample and young individual specimens measured during the study.

Table 2. The morphometric characteristics of *Ruppia brevipedunculata* from Indonesia (South Kalimantan and Rote Island) and China

Characteristic features	South Kalimantan (this study)	Rote Island (Kurniawan et al. 2024)	China (Yu and den Hartog 2014)
Plant height (cm)	20.9-69.5	-	> 40
Internode length (cm)	0.6-2.7	0.4-1.4	1-5
Leaf length (cm)	2.5-11.9	2.5-8.6	< 10-20
Leaf width (mm)	0.2-0.4	0.4-0.8	0.3-0.5
Leaf sheath length (cm)	0.4-1.6	0.5-1.6	0.5-1.5
Root length (cm)	1.6-10	1-3	1-7
Fruit beak length (mm)	0.1-0.9	-	0.3-0.5
Podogynes length (cm)	0.3-1.8	0.4-1.2	0.4-2.5
Inflorescence peduncle length (mm)	3.0-9.5	3.2-7.5	1-7
Fruit length (mm)	1.2-2.9	2.9-3.6	2.1-2.6
Fruit width (mm)	0.8-1.6	1.7-2.2	1.1-1.6
Carpel length (mm)	0.4-1.1	0.4-0.9	-
Number of carpels per flower	2 to 5	4 to 7	4

Notes: -: No data

Additionally, these size differences could result from the plant's response to habitat conditions for growth and development, such as in mariculture ponds, lagoons, estuaries, abandoned salt pans, brackish water, ditch (Yu and den Hartog 2014), and lake (Kurniawan et al. 2024). Each of these habitats has different depths. The proportion of leaf area and plant height at different depths reflects the plant's adaptation to variations in light conditions caused by turbidity or the presence of other plants (Kantrud 1991). The availability of nutrients in different habitats will affect root and rhizome length, which is crucial for nutrient uptake and survival in low-light conditions (Kantrud 1991; Strazisar et al. 2021). Furthermore, an increase in rhizome length or root biomass was observed when phosphorus was added to the sediments (Strazisar et al. 2021). Differences in salinity also lead to variations in the shape and size of the fruit in *Ruppia* plants (Kantrud 1991; Waycott et al. 2022).

Although the availability of the *R. brevipedunculata* database based on the ITS marker is still limited, the *R. brevipedunculata* from this study showed a high similarity to similar species found in GenBank. Sequence samples (South Kalimantan-Indonesia) obtained from this study were closely related to the *R. brevipedunculata* originating from China (JQ034336.1 deposited as *R. maritima* in GenBank), Rote Island-Indonesia (PQ963048.1 and PQ963047.1) and India (MW295425.1) (Figure 3). The accession number JQ034336.1 from China has been reclassified and confirmed as *R. brevipedunculata* (Yu and den Hartog 2014; Kurniawan et al. 2024). The genetic relationship between *R. brevipedunculata* from this study and those originating from China (JQ034336.1), and Rote Island-Indonesia (PQ963048.1 and PQ963047.1) can be explained by the fact that each sample has a small genetic distance value of $0.00 \pm 0.00\%$ (Table 3). This value reflects a closer genetic relationship between the two species, implying that they have fewer genetic differences. Additionally, the species from India (MW295425.1), deposited as *R. maritima* in GenBank, also shows a small genetic distance ($0.00 \pm 0.00\%$) to the *R. brevipedunculata*

species from both China and Indonesia. This indicates that those species likely share the same gene pool and show no significant intraspecific variation. Based on this, *R. maritima* from India could also be *R. brevipedunculata*.

The origin of *Ruppia* meadows in the cultivation ponds in this study is still unknown. However, the most sensible explanation that they may originate from this area as native species. This is based on the assumption that the ponds were mangrove swamps before being converted into shrimp and fish cultivation ponds, similar to that mangrove swamp in Jakarta Bay-Ancol, North Jakarta, where *R. maritima* was first found in Indonesia waters. Other possibility regarding the presence of *Ruppia* in the study area is that the seeds were brought by seabirds or the movement of seawater currents (Triest and Sierens 2013, 2014).

The waters of South Kalimantan Province are among the regions in Indonesia threatened by various factors, including anthropogenic disturbances such as wastewater from industrial palm oil operations, coal mining, and port activities (Salim et al. 2024). These factors contribute to industrial waste, such as heavy metals, and metal pollution in the waters of South Kalimantan (Kadarsah et al. 2020). Activities from oil palm plantations and coal mining have significant potential to produce heavy metal pollution. *Ruppia* plants, which are ecologically important, are known to accumulate metals (Gu et al. 2021). Therefore, the potential of *Ruppia* species, which grow abundantly in this region, could be harnessed as bioindicators and phytoremediation agents to improve the quality of water affected by various industries along the coast of South Kalimantan and other areas in Indonesia. Additionally, *Ruppia* meadows in cultivation ponds help enhance water quality by trapping particulate matter on their leaves and sediment, while absorbing nutrients in their tissues. Seagrass meadows also play a crucial role in oxygenating the water during the day, maintaining oxygen levels above the minimum required for the healthy growth of cultured animals (de los Santos et al. 2020).

Table 3. The genetic distance (%) of several *Ruppia* species originated from different regions

Sample of <i>Ruppia</i>	1	2	3	4	5	6	7	8	9	10	11	12
1		0.13	0.03	0.13	0.03	0.03	0.03	0.03	0.13	0.13	0.13	0.13
2	0.94		0.10	0.01	0.10	0.11	0.10	0.10	0.00	0.00	0.00	0.00
3	0.17	0.83		0.10	0.00	0.02	0.00	0.00	0.10	0.10	0.10	0.10
4	0.94	0.02	0.83		0.11	0.11	0.10	0.10	0.01	0.01	0.01	0.01
5	0.18	0.83	0.00	0.83		0.02	0.01	0.00	0.10	0.10	0.10	0.10
6	0.17	0.89	0.07	0.88	0.07		0.02	0.02	0.11	0.11	0.11	0.11
7	0.17	0.83	0.00	0.83	0.01	0.06		0.00	0.10	0.10	0.10	0.10
8	0.17	0.83	0.00	0.83	0.00	0.07	0.00		0.10	0.10	0.10	0.10
9	0.94	0.00	0.83	0.02	0.83	0.89	0.83	0.83		0.00	0.00	0.00
10	0.94	0.00	0.83	0.02	0.83	0.89	0.83	0.83	0.00		0.00	0.00
11	0.94	0.00	0.83	0.02	0.83	0.89	0.83	0.83	0.00	0.00		0.00
12	0.94	0.00	0.83	0.02	0.83	0.89	0.83	0.83	0.00	0.00	0.00	

Notes: Sample 1: *R. megacarpa* (JQ034337.1_China); 2: *R. maritima* (MW295425.1_India); 3: *R. maritima* (MG215620.1_Canada); 4: *R. maritima* (JN113282.1_France); 5: *R. maritima* (AB728734.1_Vanuatu); 6: *R. drepanensis* (MN958118.1_Italy); 7: *R. cirrhosa* (MG215930.1_Canada); 8: *R. cirrhosa* (JQ034335.1_China); 9: *R. brevipedunculata* (JQ034336.1_China); 10: *R. brevipedunculata* (PQ963048.1_Rote_Island-Indonesia2); 11: *R. brevipedunculata* (PQ963047.1_Rote_Island-Indonesia1); 12: *R. brevipedunculata*_ITS_South_Kalimantan-Indonesia). The genetic relationships within the same species are shown in the bold type. The numbers above the diagonal: standard error, the numbers below the diagonal: genetic distance value

In conclusion, the submerged plants from cultivation ponds in the intertidal zone of South Kalimantan were identified as seagrass, *R. brevipedunculata*, which grows mono-species. The phylogenetic tree revealed that *R. brevipedunculata* from South Kalimantan (Indonesia) have high similarities with *R. brevipedunculata* from China and identical to the species found on Rote Island (Indonesia) despite some morphometric value that distinguish them morphologically. Further research to investigate the relationship among species is necessary using two or more markers (matK, rpoB, rpoC1, trnH-psbA) from the nuclear genome.

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