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Research Article
Exploring the Distribution of *Mecardonia procumbens* (Miller) Small in Select Regions of Hanoi Region, Vietnam

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Abstract

Background and Objective: *Mecardonia procumbens* (Miller) small, a member of the Plantaginaceae family, is a plant native to tropical and subtropical regions of the Americas. This study aimed to characterize *M. procumbens* specimens collected from Hanoi, Vietnam using the morphological and genetic analysis methods. **Materials and Methods:** Sixty plant specimens were used to analysis their morphology characteristics using their vegetative and reproductive organs. Their extracted DNA was used to amplify *matK*, *rbcLa* and *trnH-psbA* regions using 390F/1326R, 1f/724r and PA/TH primers, respectively. The obtained sequences of three DNA barcodes were used for the identification of species using the BLAST program using the BOLD (barcode of life data systems) and GenBank databases. **Results:** The analysis of three DNA barcodes using BOLD and GenBank databases showed that the specimens have higher similarity compared to the *M. procumbens* species. The plant specimens in this study have 5 stamens in their flowers, showing a unique characteristic compared to the previously published genus *Mecardonia*. **Conclusion:** The DNA barcodes and morphological analysis suggested that the specimens collected in this study could be an additional species of *M. procumbens* in Vietnam.

Key words: *Mecardonia procumbens*, plant taxonomy, DNA barcoding, morphology analysis, *matK*, *rbcLa*, *trnH-psbA*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mecardonia is a plant genus that originates from South America and belongs to the Plantaginaceae family[1]. It can be found in several countries including Argentina, Chile, Bolivia, Brazil (South America), Mexico, Puerto Rico and Cuba (Central America), as well as certain parts of the USA such as Florida and Mississippi. It possesses several distinguishing characteristics such as being herbaceous and turning black when dry. The leaves are ovate and serrated, arranged opposite to each other. The flowers grow individually in the leaf axils and have stalks approximately 8-20 mm long, accompanied by bracts[2]. The calyx consists of five unequal-sized sepals, while the corolla comprises five petals and four stamens. The plants bear egg-shaped capsules and produce numerous seeds with network striations[2]. Plantaginaceae is an enlarged family that has been divided extensively due to the results of DNA sequence studies. Albach *et al.*[3] identified 47 members of Plantaginaceae and 7 outgroups based on 3561 aligned characters from four DNA regions, including ITS, *trnL-F*, *rps16* intron and *matK-trnK*[3]. The chromosome numbers and DNA contents of *Mecardonia* (Gratiolae and Plantaginaceae) were investigated by Sosa *et al.*[4]. Three ploidy levels were found suggesting that polyploidy is common and plays an important role during the evolution of this genus[4].

Mecardonia procumbens (Miller) small has been used as a medicinal plant in traditional medicine in some countries, to treat unspecified medicinal disorders, due to its high content of total phenolic, flavonoid and tannin[5]. The plant extract using different solvents at the flowering stage showed a strong antioxidant activity[5]. This plant has been considered an additional species in some African countries, such as Cameroon and Tanzania. In Asia, it has been found and characterised in several countries, such as Taiwan[6], Nepal[7] and Singapore[8].

However, there have been no reports of this species in Vietnam. In Vietnam, the presence of the Plantaginaceae family is solely recorded by the genus *Plantago*, which encompasses two species. During a survey conducted in the vegetable-growing area of Thuong Tin district, Hanoi, an unidentified plant species was discovered, exhibiting the following traits: A cow-like appearance, ovate leaves with serrated edges arranged opposite to each other, staggered flower buds, flower stalks approximately 2 cm long, yellow flowers with burgundy streaks on the petals and a blackened fruit when dry, accompanied by the presence of a calyx. After careful identification and analysis, it was determined that the characteristics of this species closely resemble those of *Mecardonia procumbens* (Miller) small. Therefore, this discovery is considered a new species recorded for Vietnam, adding to the existing species in the country.

The species was found in organic vegetable fields and abandoned vegetable fields, which had been cultivated for a few years and were covered with various vegetables and weeds. These fields were located at an altitude of 0-12 m above sea level, featuring moist soil and being situated under the canopy of other species. Specimens were collected from two locations: (1) Organic vegetable beds in a white net house, where cruciferous vegetables and nettles were grown, using mesh 16 for the roof and mesh 20 for the surroundings or edges and (2) Partially shaded wildland, coexisting with other species. The plants were predominantly found in position (1), with no individuals observed in the vegetable bed, likely due to weed management measures implemented by farmers. Therefore, the present study was performed to investigate the *M. procumbens* specimens collected from Vietnam using the methods of morphological and genetic analysis.

MATERIALS AND METHODS

Study area: The laboratory work was carried out at the Laboratory of Molecular Biology and Laboratory of the Department of Botany, Faculty of Biology at Hanoi National University (HNUE), Vietnam from December, 2021 to November, 2022.

Plant sampling: The plant samples in this study were collected from several locations, including Le Loi commune, Tan Minh commune and Van Phu commune in Thuong Tin District, Hanoi, Vietnam from November, 2021 to October, 2022. The species is colonic and scattered so a branch is taken from each individual. Samples with lengths from 8 to 15 cm must have a complete set of root, stem, leaf, flower and fruit. Sixty plant samples were grown in the laboratory of the Department of Botany, Faculty of Biology, HNUE under regular conditions after being collected from the field. Samples used for anatomical analysis were preserved in 50% alcohol as soon as collected.

Morphological analysis: The vegetative and reproductive organs of the plant samples were used for morphological analysis following studies by researchers[9,10].

Table 1: Sequences of primers for each barcode used in this study

Barcodes	Primers	Sequence (5' to 3')
matK	390F	CGATCTATTCATTCAATATTTC
	1326R	TCTAGCACACGAAAGTCGAAGT
rbcLa	1f	ATGTCACCACAAACAGAAAC
	724r	TCGCATGTACCTGCAGTAGC
trnH-psbA	PA	GTTATGCATGAACGTAATGCTC
	TH	CGCGCATGGTGGATTCACAATCC

Table 2: Conditions of thermal cycles for amplification of the barcodes used in this study

		Barcodes		
Number of cycles	Steps	*matK*	*rbcLa*	*trnH-psbA*
1	Initial denaturation		98°C, 30 sec	
35	Denaturation		98°C, 10 sec	
	Annealing	52°C, 20 sec	55°C, 30 sec	64°C, 30 sec
	Extension	72°C, 50 sec	72°C, 1 min	72°C, 40 sec
1	Final extension		72°C, 10 min	

The vegetative organs of the plant samples were used to prepare the microscopic slides. Briefly, 12% Javen, 3% HCl, 0.1% Green-Methyl and 7% Carmine were used to stain the microscopic samples. The slides with stained samples were observed and photographed using a computer-connected microscope system (ZEISS Axio Scope A1 with Axiocam 105 Color) and ZEN Microscopy software 2.6 (Carl Zeiss, Oberkochen, Germany).

DNA extraction and PCR: The fresh young leaves of the plant samples were used to extract total DNA. The total DNA of the plant sample was extracted using Genomic DNA Isolation Kit (NORGEN Biotek, Ontario, Canada) according to the manual of the manufacturer. The isolated DNA was checked by using 1% agarose gel electrophoresis and a nanodrop machine (GE healthcare).

Three DNA barcodes were used to identify the species of the specimens at the molecular level, including *matK* (Maturase K), *rbcLa* (Ribulose-bisphosphate carboxylase) and chloroplast non-coding DNA region *trnH-psbA*[11,12].

The PCR reactions were performed using three primer pairs corresponding to three different DNA barcode regions as shown in Table 1[12]. The PCR amplifications were carried out to target specific DNA regions, utilizing Phusion™ High-Fidelity DNA Polymerase (2 U μL^{-1}) from Thermo Fisher Scientific, USA. The components of PCR reactions were prepared as instructed by the provider and the PCR running programs for each barcode were shown in Table 2.

Following PCR amplification, purification of PCR products and DNA samples was accomplished either directly or through 1% agarose gel electrophoresis. This purification process utilized the GenElute™ PCR Clean-Up Kit or the GenElute™ Gel Extraction Kit (Sigma-Aldrich). The DNA quantification was conducted using the SimpliNano™ Spectrophotometer (Biochrom, Harvard Bioscience, UK). The purified PCR products were sequenced using the Sanger sequencing service of First Based Sequencing (Malaysia).

Bioinformatics analysis: To identify the highly similar species, the sequences of the PCR products obtained from the sequencing service were used to run the online Nucleotide BLAST program (Basic Local Alignment Search Tool), using two databases, including NCBI (National Center for Biotechnology Information)[13] and BOLD Identification System (IDS)[14]. The list of the highest similar species was obtained for further analysis.

Statistical analysis: The samples in this study were evaluated at 3 replicates and presented as Mean±Standard Deviation (SD). The DNA sequences were checked and corrected by BioEdit 7.0 software to check the peak of nucleotides and remove the unreadable nucleotides at both ends of the PCR products.

RESULTS

DNA barcoding analysis: The sequences of three DNA barcodes obtained from this study were used for barcoding analysis (Supplementary document). The analysis was performed using the BLAST tool based on two different databases. Based on the NCBI database, the results of BLAST analysis show that the DNA barcode sequences of this species have the highest similarity with different species. Specifically, for the *matK* DNA barcode, the highest similarity reaches 97.4% with *Mecardonia procumbens*. For *rbcLa* barcode, the highest similarity is 98.34% with *Jacaranda brasiliana*. As for the *trnH-psbA* barcode, the highest similarity is 100% with

Table 3: BLAST results of species identification using two different databases

	BLAST results					
	BOLD			GenBank (NCBI)		
Barcodes	Scientific name	E value	Similarity (%)	Scientific name	E value	Identity (%)
matK	*Halleria tetragona*	0	92.32	*Mecardonia procumbens*	0	97.40
	Schlegelia fuscata	0	92.32	*Schlegelia fuscata*	0	92.33
	Teedia lucida	0	92.20	*Halleria tetragona*	0	92.33
rbcLa	*Jacaranda arborea*	0	98.34	*Jacaranda brasiliana*	0	98.34
	Jacaranda sparrei	0	98.34	*Jacaranda arborea*	0	98.34
	Jacaranda mimosifolia	0	98.17	*Jacaranda sparrei*	0	98.34
trnH-psbA	NA	NA	NA	*Capraria biflora*	0	100.00
	NA	NA	NA	*Magnoliophyta* sp.	1.00E-176	98.86
	NA	NA	NA	*Mecardonia acuminata* subsp. *peninsularis*	8.00E-89	80.87

NA: Not available and E value: Expect value

Capraria biflora (Table 3). Based on the BOLD database which is only 2 available barcodes, including *matK* and *rbcL*, but not for *trnH-psbA*. In this database, the two highest similarity species identified were *Halleria tetragona* and *Jacaranda arborea* with a similarity of 92.32% (*matK*) and 98.34% (*rbcLa*), respectively (Table 3).

Taken together, combined with morphological data analysis, the collected specimens in this study were confirmed belonging to the species *Mecardonia procumbens*. This is the first record of this species in Vietnam.

Habitat: The plant usually lives in a moist environment, partially shaded by taller species such as *Physalis angulata* L., *Acmella paniculata* (Wall. ex DC.) R.K. Jansen, *Cyclosorus parasiticus* (L.) Fawell., Brassicaceae family cultivated plants inside a white mesh greenhouse with a mesh size of 16 (roof) and mesh size of 20 (sides).

Individuals are commonly found randomly distributed in furrows of vegetable fields or along field edges. No individuals were found on the vegetable beds as the studied species, which is a creeping plant, struggles to compete for light with other cultivated plants such as *Basella alba* or Brassicaceae (these species have enclosed canopy). Additionally, it is eliminated by weed management measures.

Morphology and structure

Stems: The plant has a creeping stem that eventually stands upright. In the early stages of development, the procambium layer operates intermittently to produce discrete bundles in the phloem order outside the xylem. Xylem fibers and the Casparian strip have not yet formed (Fig. 1a). The stem branches out early from the base, with a smooth stem that is devoid of hair. Each node gives rise to adventitious roots that penetrate into the ground. The secondary roots exhibit growth (Fig. 1b, c), with the root diameter increasing over time. The stem has a square cross-section (Fig. 1d). The average length of internodes during the summer growing season is 22.03±1.51 mm.

The shoot development of the species differs depending on the presence of flower buds. In leaves with developing flower buds, axillary shoots also develop, while in leaves opposite to them, both types of shoots are absent. In positions without flower buds, the axillary shoots remain in a dormant state. The flower shoots grow sporadically along the stem in a zigzag pattern (Fig. 1e).

The plant has a small stem, with limited diameter increase due to weak activity of the cambium layer. The xylem and phloem are arranged in concentric rings. Xylem and phloem rays are stable, ranging from 1 to 3 rows, extending into the parenchyma. The fiber outside the conducting system is poorly developed, with the sporadic occurrence of wood fiber cells scattered in the phloem. The thin bark consists of soft tissue with numerous small gas-filled cavities, indicating adaptation to a moist soil environment. The innermost layer, the endodermis, possesses characteristic Casparian strips typical of dicot plants. The epidermis persists on the stem for a long time (Fig. 1f).

Roots: The secondary root system develops strongly. Secondary xylem and phloem are the main components of the roots, with the xylem fibers developing prominently. The Casparian strip persists for a long time due to the weak activity of the cambium layer and the primary cortex also persists for a considerable period within the root structure. The primary cortex consists of a soft tissue arrangement that forms large gas-filled cavities, occupying a significant proportion of the cortex area. The secondary cortex appears later, with the cork cambium originating from the layer of soft tissue cells just below the epidermis (Fig. 1b, c). In the primary root, an average of 3 xylem poles differentiates.

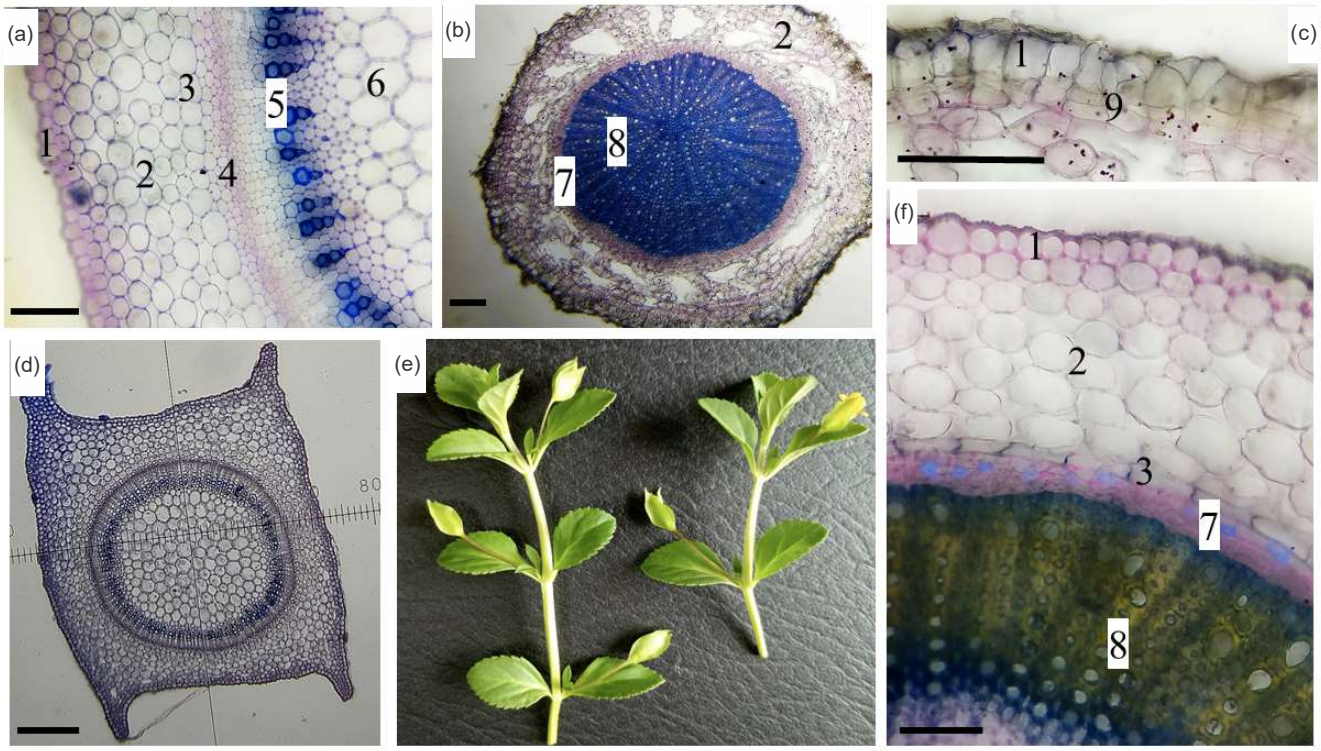

Fig. 1(a-f): Structural characteristics of stem, (a) Partial cross-section structure of the primary stem, (b) Structure of the secondary root, (c) Structure of the root cortex, (d) Cross section of the stem, (e) Partial morphological structure of the stem and (f) Partial cross-section structure of the secondary stem

Scale bar: 100 μm, 1: Epidermis, 2: Cortex parenchyma, 3: Endodermis, 4: Primary phloem, 5: Primary xylem, 6: Core of parenchyma, 7: Metaphloem, 8: Metaxylem and 9: Phellogen

Leaves: The leaf is ovoid-shaped, simple and entire, with evenly serrated leaf margins. It has pinnate veins that grow opposite each other, with a smooth leaf surface and a short petiole. The size of the leaves in the axils with developing flower buds is larger than those without flower buds on the opposite side, arranged in alternating pairs. The average size is 19.63±1.05×11.68±0.85 mm and 16.70±1.11×9.02±0.68 mm.

The leaf is slightly fleshy and succulent due to the development of the parenchyma tissue system. The epidermal cells on the upper surface are usually larger than those on the lower surface (Fig. 2a-d). The stomata are bean-shaped and subsidiary cells are present on both leaf surfaces. The main veins have a similar structure to the young stem, with only 1-2 layers of thickened tissue cells beneath the epidermis. Hard tissue surrounding the vascular bundles is almost absent. In the leaf blade, the palisade and spongy tissues are differentiated, typically with a single layer of palisade cells, while the spongy tissue is arranged to form large air-filled cavities. The bundle sheath does not contain chloroplasts (Fig. 2e).

Reproductive organs: The flowers are solitary, growing in the leaf axils, with a round, smooth flower stalk that is approximately the same length as the leaf. Each flower has two small strap-shaped sepals (Fig. 2f). Flowers staggered along the stems (Fig. 3a). The flower has 5 petals and 5 sepals, which are separate and form two whorls. The outer whorl consists of 3 larger elliptical-shaped segments (7.73±0.25×3.75±0.15 mm) with pointed tips, while the inner whorl consists of 2 filamentous sepals, measuring about 7 mm in length and less than 1 mm in width (Fig. 3b). The sepals persist long after fruit formation. The petals are fused together to form a tube and the throat of the flower tube has numerous developed glandular hairs. The flowers are yellow and the lip petals have reddish-brown to purple streaks (Fig. 3c, d). There are 5 stamens, with only the filaments attached to the petals and the anthers are adnate to the back (Fig. 3e, f). The pollen grains are smooth, rounded to slightly elliptical, with a pollen diameter of 17.5 μm. The presence of 5 stamens is a distinguishing feature from previous publications (Fig. 3e, f).

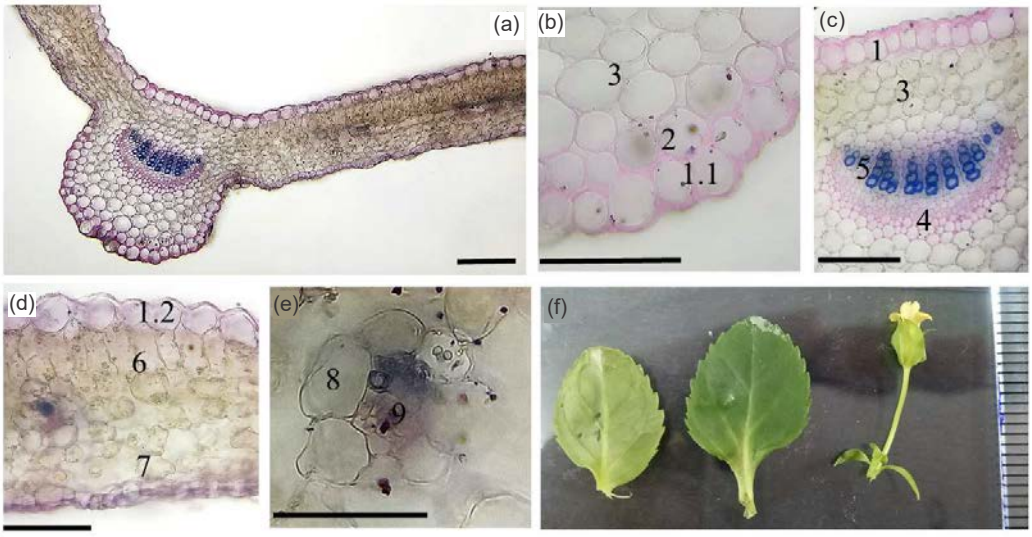

Fig. 2(a-f): Morphology and structure characteristics of leaf, (a) Cross-section of the leaf, (b, c) Partial structure of leaf veins, (d) Partial structure of leaf blade, (e) Vascular bundle sheath and (f) Leaf morphology

Scale bar: 100 μm, 1: Epidermis, 1.1: Epidermis on the underside of leaf veins, 1.2: Epidermis on the blade leaf, 2: Collenchyma in the leaf vein, 3: Parenchyma, 4: Phloem, 5: Xylem, 6: Chlorenchyma, 7: Vacant parenchyma, 8: Surrounding vascular bundle cells and 9: Vascular bundle

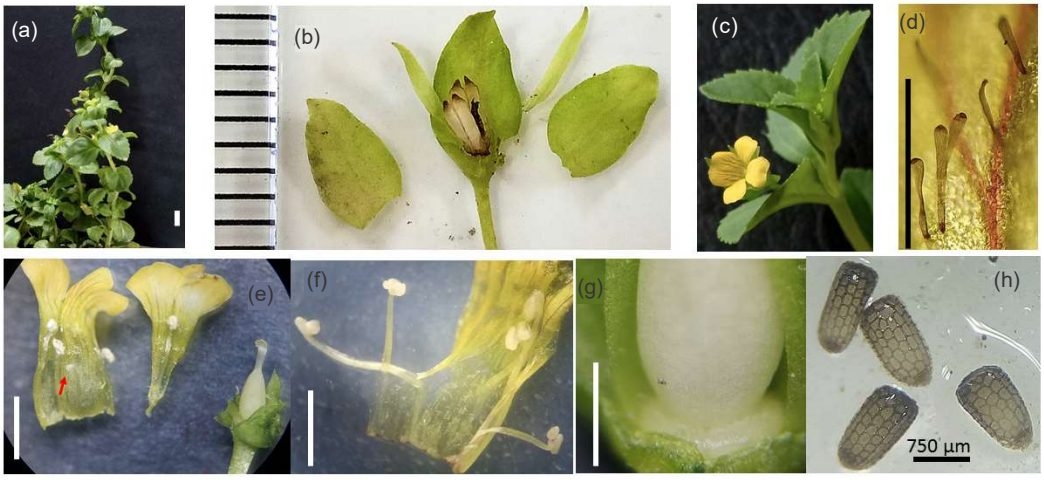

Fig. 3(a-h): Structural characteristics of flower, (a) Partial structure of flowering stem, (b) Dehiscent dry fruit, (c) Flower morphology, (d) Glandular hairs in the corolla tube, (e) Flower structure, (f) Each flower with 5 stamens attached to the corolla tube, (g) Nectar gland at the base of the ovary and (h) Network like structural pattern

Scale bar: 3 mm and H: Seed

The upper ovary is approximately 1.5 mm long with an approximate diameter of 0.5 mm. It consists of two fused carpels, forming two locules. The ovary wall is white and the ovules are attached to the central placenta, with numerous ovules present. The nectar disc is located at the base of the ovary (Fig. 3g). The mature fruit is dry and ellipsoid-shaped, with a length of about 5-6 mm and a width of 2 mm. It dehisces longitudinally through a fissure (Fig. 3b). As the fruit ripens, it gradually changes from green to black. The seeds are dark brown, measuring 0.875×0.375 mm and when immature, the seeds are translucent white. The surface of the seed has a network-like structural pattern (Fig. 3h).

Flower formulation:

$$\uparrow \mathrm{K}_5\mathrm{C}_5\mathrm{A}_5\underline{\mathrm{G}}_2$$

The floral structure indicates a bisexual flower, symmetrical on both sides, with a five-parted calyx, the corolla has five fused petals, five stamens, a superior ovary and two fused carpels.

DISCUSSION

This present study is the first report of the *M. procumbens* found in Vietnam with distinguished characteristics of its reproductive organ. The molecular data of DNA barcoding has been used for species identification recently[11,12]. To precise identification, three different barcodes have been used in this study, including *matK*, *rbcLa* and *trnH-psbA*. In which, *matK* is one the most important barcodes, due to its higher discrimination success compared to the *rbcL*, More *et al*.[15] applied *matK* to identify correctly 46 out of 60 plant species at genus and species level using their own generated signatures.

In this study, the highest similarity between the collected specimens compared to *Capraria biflora* (100%) and *Magnoliophyta* sp. (98.86%) corresponding to the *trnH-psbA* barcode and *Jacaranda* sp. (98.34%) corresponding to the *rbcLa* barcode and *Mecardonia procumbens* (97.40%) corresponding to *matK* barcode (Table 3). However, *Capraria biflora*, *Magnoliophyta* sp. and *Jacaranda* sp., have completely different morphological characteristics compared to the collected samples in this study. Therefore, the present specimens were identified as *M. procumbens*, due to a combination of genetic and morphological analyses. These results have confirmed that using only the molecular genetic data in plant species identification is not sufficient, because incongruence between morphological and molecular markers has been reported[16]. Therefore, it should combine with the analysis of morphological characteristics.

Most of the morphological characteristics of the *M. procumbens* samples collected in this study are similar to other published documents[2,6-8,10]. However, there are several different points, such as the growth pattern of this species in Vietnam is distinct from the shoot development observed in previous studies conducted in Taiwan[6]. In addition, *M. procumbens* is generally categorized as a wetland plant in the USA, according to Lichvar *et al*.[17]. It is classified as a facultative wetland hydrophyte, meaning it typically occurs in wetland environments but can also be found in non-wetland areas, in regions other than the atlantic and gulf coastal plains (AGCP). In the AGCP, it is considered an obligate wetland hydrophyte, meaning it almost always occurs in wetlands. However, some of the records from disturbed locations in the AGCP, including the Warren County site, may lack the typical characteristics of wetland environments, such as wetland hydrology, hydrophytic vegetation and hydric soils. These findings indicate the need for a reassessment of *M. procumbens'* wetland indicator status within the AGCP region. It is possible that *M. procumbens* should be regarded as a facultative wetland hydrophyte in this specific area[2]. Nevertheless, the *M. procumbens* samples analyzed in this study were sourced from non-wetland vegetative areas. Nonetheless, the diverse phenotypic traits exhibited by these specimens suggest the possibility of unique ecological and metabolic features in comparison to previous research.

The combination of morphological and sequence analysis of several DNA regions indicated that the specimens found in this study belonged to the species *M. procumbens*, based on both general BOLD and GenBank (NCBI) reference databases. The identification of *M. procumbens* in Vietnam is documented here for the first time. This discovery represents a novel occurrence of the species within the country. Notably, this particular population of *M. procumbens* exhibits a distinctive phenotypic trait related to its reproductive organs. Specifically, each flower is observed to possess 5 stamens attached to the corolla tube (Fig. 3), contrasting with previous reports indicating 4 stamens per flower in this species[2,6]. This notable difference in stamen count suggests the possibility of a potential new species that has evolved within the genus. The reasons behind the presence of 5 stamens in the flowers and the evolutionary mechanisms responsible for this trait remain unclear. Further investigation through molecular genetics and systematic analysis is necessary to confirm these findings and address the unanswered questions surrounding this unique characteristic.

RECOMMENDATIONS

The plant specimens uncovered in this study have unveiled intriguing possibilities. Further investigations into the chromosome and genome sequences, coupled with the analysis of chemical composition and biological attributes of plant extracts derived from this species, offer substantial potential for the future. These extensive explorations may shed light on the evolutionary connections between the plant specimens collected in Vietnam and those originating from different regions. Moreover, the expansive application of these plant extracts to tackle diverse human ailments presents a noteworthy path for exploration.

CONCLUSION

Morphological and genetic analyses have confirmed the presence of *M. procumbens* in certain vegetable cultivation areas in Hanoi, Vietnam. This is the first recorded occurrence of this species in Vietnam. The *M. procumbens* specimens have 5 stamens in the flower, which distinguishes them from the characteristics of both *M. procumbens* and the previously published genus *Mecardonia*. This discovery implies the potential for this to be a new species that has evolved within the documented genus in Vietnam.

SIGNIFICANCE STATEMENT

Mecardonia procumbens (Miller) small, belonging to the Plantaginaceae family, is found in several countries in South America, Central America and is an additional species in some countries in Africa and Asia. However, it has not been reported in Vietnam. This study described its morphological, anatomical, genetic characteristics and distribution. Results confirmed the presence of *M. procumbens* in Vietnam, adding this species to the Vietnamese plant system, with a genetic similarity of 97.4% to data from *M. procumbens* in BOLD and GenBank. The pentamerous stamen is a distinguishing feature of *M. procumbens* individuals found in Vietnam.

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REFERENCES

1. López, R., J. Villanueva and J. Antonio, 1794. Flora of Peru, and the Prodromus of Chile [In Latin]. Madrid: at Sancha's printing house, Calle de Sta Engracia, Spain, Pages: 153.
2. Philley, K.D., 2020. *Mecardonia procumbens* (plantaginaceae) new to the flora of mississippi. Phytoneuron, Vol. 80.
3. Albach, D.C., H.M. Meudt and B. Oxelman, 2005. Piecing together the "new" Plantaginaceae. Am. J. Bot., 92: 297-315.
4. Sosa, M.M., M.B. Angulo, J.A. Greppi and V. Bugallo, 2016. Chromosome numbers and DNA content in some species of *Mecardonia* (Gratiolae, Plantaginaceae). Comp. Cytogenet., 10: 769-780.
5. Neglur, C.B. and A.M. Gajakosh, 2021. Total phenolic, flavonoid, tannin contents, and antioxidant activity of organic extracts of vegetative and flowering stages of *Mecardonia procumbens* (Mill.) Swall. Asian J. Pharm. Clin. Res., 14: 84-86.
6. Chen S.H. and M.J. Wu, 2001. Notes on two newly naturalized plants in Taiwan. Taiwania 46: 85-91.
7. Joshi, N.P., 2021. Ecological and ethnobotanical values of weeds found in the spring rice fields in Chitwan, Nepal. Ethnobotany Res. Appl., Vol. 22. 10.32859/era.22.37.1-19.
8. Chen, L.M.J., B.C. Ho, L.M. Choo and S.L. Koh, 2018. Additions to the Flora of Singapore, new and overlooked records of naturalised plant species (1). Gardens' Bull. Singapore, 70: 91-101.
9. Ornduff, R., 1978. Reproductive characters and taxonomy. Syst. Bot., 3: 420-427.
10. Halloy, S., 1990. A morphological classification of plants, with special reference to the New Zealand alpine flora. J. Veg. Sci., 1: 291-304.
11. Costion, C., A. Ford, H. Cross, D. Crayn, M. Harrington and A. Lowe, 2011. Plant DNA barcodes can accurately estimate species richness in poorly known floras. PLoS ONE, Vol. 6. 10.1371/journal.pone.0026841.
12. Loera-Sánchez, M., B. Studer and R. Kölliker, 2020. DNA barcode *trnH-psbA* is a promising candidate for efficient identification of forage legumes and grasses. BMC Res. Notes, Vol. 13. 10.1186/s13104-020-4897-5.
13. Johnson, M., I. Zaretskaya, Y. Raytselis, Y. Merezhuk, S. McGinnis and T.L. Madden, 2008. NCBI BLAST: A better web interface. Nucl. Acids Res., 36: W5-W9.
14. Ratnasingham, S. and P.D.N. Hebert, 2007. BOLD: The barcode of life data system (http://www.barcodinglife.org). Mol. Ecol. Notes, 7: 355-364.
15. More, R.P., R.C. Mane and H.J. Purohit, 2016. matK-QR classifier: A patterns based approach for plant species identification. BioData Min., Vol. 9. 10.1186/s13040-016-0120-6.
16. Duminil, J. and M. di Michele, 2009. Plant species delimitation: A comparison of morphological and molecular markers. Plant Biosyst.-Int. J. Dealing Aspects Plant Biosyst., 143: 528-542.
17. Lichvar, R.W., D.L. Banks, W.N. Kirchner and N.C. Melvin, 2016. The national wetland plant list: 2016 wetland ratings. Phytoneuron, Vol. 30.