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Original Article

DNA barcoding confirms the identity of the invasive *Sonchus arvensis* in Java, Indonesia

Código de barras de DNA confirma a identidade da espécie invasora *Sonchus arvensis* em Java, Indonésia

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Abstract

Sonchus arvensis subsp. *arvensis* is a perennial plant that serves both as a traditional medicinal herb and a prolific invasive weed. Its recent introduction to Southeast Asia, including Java, Indonesia, poses a potential threat to native biodiversity, yet its genetic provenance and invasion history in the region are uncharacterized. To provide a reliable species-level identification, we employed DNA barcoding of the chloroplast genes *rbcL* and *matK*. Phylogenetic analysis revealed that samples from the four geographically distinct areas were genetically uniform based on these markers and were placed within a clade containing Eurasian *S. arvensis* accessions. The invader was distinct from the native Australasian *Sonchus* species. This work represents the first molecular confirmation of *S. arvensis* in Java using DNA barcodes. While it establishes species identity, further genomic studies are required to resolve the population history, introduction pathway, and ecological impact of this invasive species.

Keywords: invasive species, conservation genetics, chloroplast DNA, Asteraceae.

Resumo

Sonchus arvensis subsp. *arvensis* é uma planta perene que serve tanto como erva medicinal tradicional quanto como erva daninha invasora prolífica. Sua recente introdução no Sudeste Asiático, incluindo Java, Indonésia, representa uma ameaça potencial à biodiversidade nativa, porém sua origem genética e histórico de invasão na região ainda não foram caracterizados. Para fornecer uma identificação confiável em nível de espécie, empregamos o código de barras de DNA dos genes cloroplastídicos *rbcL* e *matK*. A análise filogenética revelou que as amostras das quatro áreas geograficamente distintas eram geneticamente uniformes com base nesses marcadores e foram posicionadas dentro de um clado contendo acessos eurasiáticos de *S. arvensis*. A espécie invasora era distinta das espécies nativas de *Sonchus* da Australásia. Este trabalho representa a primeira confirmação molecular de *S. arvensis* em Java usando códigos de barras de DNA. Embora estabeleça a identidade da espécie, estudos genômicos adicionais são necessários para elucidar o histórico populacional, a via de introdução e o impacto ecológico dessa espécie invasora.

Palavras-chave: espécies invasoras, genética da conservação, DNA de cloroplastos, Asteraceae.

1. Introduction

Sonchus arvensis Linnaeus subsp. *arvensis* (perennial sow-thistle) is a vigorous, deep-rooted, perennial herbaceous member of the family Asteraceae, found growing wild across temperate Eurasia. Beyond its native range, it is a successful invader, widely naturalized and considered a noxious weed in North America and Australia (Masana et al., 2020). Its recent spread into Southeast Asia, including Indonesia, poses a potential threat to local biodiversity and agriculture (Wahyuni et al., 2019). In Java, *S. arvensis* is found in disturbed environments, thriving along roadsides, in fields, and in riparian zones, where its competitive growth and adaptability allow it to dominate. It is capable of both vegetative and prolific seed reproduction, with a single plant producing up to 60,000 seeds (Lemna and Messersmith, 1990), facilitating its rapid spread in new territories.

Despite its status as an invasive weed, in Indonesia, the plant (known locally as *tempuyung*) is also utilized in traditional medicine, decocted to treat swellings, kidney stones, and hypertension (Wahyuni et al., 2019; Jayani et al., 2020). Evidence from bioactivity-guided studies confirms the presence of therapeutic phytochemicals, e.g., sesquiterpene lactones, phenolic acids, and flavonoid glycosides that harbor anti-inflammatory, antihypertensive, and antioxidant effects (Parisi et al., 2024; Kim et al., 2007). This dual role as both an invasive threat and a medicinal

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resource creates a complex management challenge, necessitating accurate identification.

This challenge is compounded by extensive morphological similarities within the genus *Sonchus*. Specifically in Java, *S. arvensis* is difficult to distinguish from both introduced congeners like *S. oleraceus* and *S. asper*, and from native Javanese species based on leaf morphology, latex, and floral structures alone (Boulos, 1973; Mejías and Valdés, 2004; Mejías et al., 2018). Furthermore, *S. arvensis* itself comprises different ploidy levels: tetraploid (2n = 36) and hexaploid (2n = 54) (Boulos, 1973), suggesting divergent evolutionary lineages with potentially varying invasive capacities (Krahulcová, 2003). These taxonomic ambiguities underline the critical need for molecular approaches to ensure accurate identification, which is the first step in any effective management or conservation strategy.

DNA barcoding, using the chloroplast genes *rbcL* and *matK* as recommended by the Consortium for the Barcode of Life (Hollingsworth et al., 2009), provides an efficient tool for such identification. The combination of the highly universal *rbcL* and the more variable *matK* has proven effective for species-level resolution in many plant groups (Hollingsworth et al., 2009; Fazekas et al., 2008). In *Sonchus*, these markers have helped clarify phylogenetic relationships, revealing, for instance, that annual weedy species are more closely related to insular woody ones from the Canary Islands than to mainland perennials (Kim et al., 2007).

While complete chloroplast genome sequencing of *S. arvensis* has provided a robust taxonomic framework within the tribe Cichorieae (Cho et al., 2019; Wang et al., 2021; Masana et al., 2020), the utility of the standard barcode loci (*rbcL* and *matK*) for pinpointing the geographic origin of invasive populations and delineating them from native relatives at a regional level remains to be fully explored. This is a crucial application for invasion biology.

For an invasive species, understanding the genetic provenance of an introduced population can reveal its invasion history (e.g., single vs. multiple introductions), predict its ecological behavior, and inform biocontrol strategies. Furthermore, confirming the genetic distinctness of an invasive from native species is essential for assessing risks such as competition and hybridization, which are central to conservation efforts.

Therefore, this study moves beyond simple identification. We employ phylogenetic analysis of *rbcL* and *matK* to achieve a definitive molecular identification of the *Sonchus* populations in Java and to place them within a global phylogenetic context. We aim to: (1) confirm the taxonomic identity of the Javanese populations as *S. arvensis*, and (2) assess their phylogenetic relationship with global accessions and native Australasian relatives. We note that the conserved nature of these chloroplast markers limits their utility for inferring introduction pathways or population-level processes; their primary utility here is for accurate species determination.

2. Material and Methods

2.1. Plant sampling and identification

Leaf samples of *Sonchus arvensis* L. were collected from four geographically separate populations across Java Island, Indonesia, to enable genetic identification. Sampling sites were: Kletak Hamlet, Tutur District, Pasuruan Regency, East Java (Voucher ID: SA.P/1/05/2025), Kalisoro Village, Tawangmangu District, Karanganyar Regency, Central Java (Voucher ID: SA.K/2/05/2025), Garbosari Village, Samigaluh District, Kulon Progo Regency, Special Region of Yogyakarta (Voucher ID: SA.Y/3/05/2025), and Diwak Village, Bergas District, Semarang Regency, Central Java (Voucher ID: SA.S/4/05/2025) (see Figure 1).

Healthy leaf tissue was collected from multiple pre-reproductive (approximately two-month-old) individuals at each site. Specimens were identified in the field by a certified botanist, Mr. Dwi Narko (Purwodadi Botanical Garden, Pasuruan, Indonesia). Voucher specimens were deposited in the herbarium of the Biosystematics Laboratory, Department of Biology, Airlangga University, Surabaya, Indonesia.

2.2. DNA extraction and quantification

Total genomic DNA was extracted from 80 mg of *Sonchus arvensis* L. leaf tissue using the Plant Genomic DNA Kit (Tiangen, China). The extraction was done according to the manufacturer›s instructions. The quality and integrity of the extracted DNA were assessed with 1% agarose gel electrophoresis (Promega, USA) and visualized under a UV transilluminator.

2.3. PCR amplication and sequencing

Two plastid DNA barcode regions, *rbcL* and *matK*, were amplified by polymerase chain reaction (PCR) using the following primers: *rbcL*: 5'-AAGTTCCTCCACCGAACTGTAG-3′ (forward) and 5′ -TACTGCGGGTACATGCGAAG-3′ (reverse) and *matK*: 5'-TGGTTCAGGCTCTTCGCTATTG-3' (forward) and 5'-CTGATAAATCGGCCCAAATCGC-3′ (reverse). The volume of the PCR reactions was 35 µL, consisting of 17.5 µL GoTaq® Green Master Mix, each primer between 350 and 500 µM in concentration, 50 ng/µL of DNA template, and the volume was made up with nuclease-free water. The thermal cycling was done in an Eppendorf® Mastercycler Personal, with an initial denaturation of 94°C for 5 minutes; denaturation at 94°C for 30 seconds, and then annealing at 56°C for 45 seconds, and extension at 72°C for 45 seconds in a total of 35 cycles; and a final 5-minute extension at 72°C. PCR products were confirmed using 1.5% agarose gel electrophoresis in 0.5X Tris-Acetate-EDTA (TAE) buffer (Promega, USA). The products successfully amplified were scaled up to 50 µL and sent for sequencing at Macrogen Inc. (Korea).

2.4. Sequence and phylogentic analysis

Consensus sequences for each sample were assembled from forward and reverse reads using BioEdit v7.2.5 (Hall, 1999). Initial taxonomic identification was confirmed using BLASTn searches against the NCBI Nucleotide database (Altschul et al., 1990). For phylogenetic reconstruction, our newly generated sequences (see Table 1 for GenBank accession numbers) were aligned with a globally representative set of *Sonchus* reference sequences from GenBank, including accessions from the native Eurasian range, other introduced ranges, and native

Figure 1. *Sonchus* species collected for DNA barcoding analysis using *rbcL* and *matK* gene markers. *Sonchus arvensis* specimens collected from four locations: (A) Kletak, (B) Tawangmangu, (C) Yogyakarta, and (D) Semarang.

Table 1. Voucher information and GenBank accession numbers for *Sonchus arvensis* subsp. *arvensis* sequences generated in this study.

Population (Location)	Voucher ID	Gene	GenBank Accession Number
Kletak, East Java	SA.P/1/05/2025	*matK*	PX409517
Kletak, East Java	SA.P/1/05/2025	*rbcL*	PX409521
Semarang, Central Java	SA.S/4/05/2025	*matK*	PX409518
Semarang, Central Java	SA.S/4/05/2025	*rbcL*	PX409522
Tawangmangu, Central Java	SA.K/2/05/2025	*matK*	PX409519
Tawangmangu, Central Java	SA.K/2/05/2025	*rbcL*	PX409523
Yogyakarta, Special Region	SA.Y/3/05/2025	*matK*	PX409520
Yogyakarta, Special Region	SA.Y/3/05/2025	*rbcL*	PX409524

Australasian species. *Prenanthes* sp. was selected as the outgroup. Multiple sequence alignment was performed with ClustalW (Thompson et al., 1994) implemented in MEGA7 (Kumar et al., 2016).

Phylogenetic relationships were inferred using the Neighbor-Joining (NJ) method based on the Maximum Composite Likelihood model (Tamura et al., 2004). The robustness of the tree topology was evaluated with 1000 bootstrap replicates. All analyses were conducted in MEGA7.

3. Results

3.1. BLAST and phylogenetic analysis confirm the identity of Javanese S. arvensis

To determine the genetic identity of the *Sonchus* populations in Java, we sequenced and analyzed the plastid barcode loci *rbcL* and *matK* from populations across four geographically distinct locations on the island (Klethak, Semarang, Tawang Mangu, and Yogyakarta). BLASTn analysis of the *rbcL* gene

revealed exceptionally high sequence similarity (99.8-100%) between our Javanese accessions and several species within the genus *Sonchus* (Table 2, Figure 2). Sequences from the Klethak and Semarang populations were 100% identical to *Sonchus boulosii* (accessions NC_042244.1, MK016665.1). All populations, including Tawang Mangu and Yogyakarta, also showed 99.8% identity with *S. arvensis*, *S. asper*, *S. oleraceus*, and *S. kirkii*, among other taxa. While the Yogyakarta samples showed marginally lower query coverage (99%), all alignments had E-values of 0.0, confirming highly significant matches. Analysis of the more variable *matK* gene corroborated these findings, with all Javanese sequences showing highest identity (99.68–99.89%) to various *S. arvensis* accessions from its global range (e.g., DQ508001.1, NC_054161.1) and closely

Table 2. BLAST results of *rbcL* gene sequences from *Sonchus arvensis* ssp. *arvensis* populations in Java Island.

Species	Population	Max score	Total score	Query cover	E-value	Per. Ident
Sonchus boulossi NC 042244.1	Klethak	911	911	100%	0	100.0%
Sonchus asper MK371016.1	Klethak	905	905	100%	0	99.8%
Sonchus oleraceus MN601475.1	Klethak	905	905	100%	0	99.8%
Sonchus kirkii KT626829.1	Klethak	905	905	100%	0	99.8%
Sonchus arvensis NC_054161.1	Klethak	905	905	100%	0	99.8%
Sonchus boulossi MK016665.1	Semarang	911	911	100%	0	100.0%
Sonchus asper MK371015.1	Semarang	905	905	100%	0	99.8%
Sonchus arvensis MN601474.1	Semarang	905	905	100%	0	99.8%
Sonchus oleraceus KM360989.1	Semarang	905	905	100%	0	99.8%
Dendroseris pinnata NC_051926.1	Semarang	905	905	100%	0	99.8%
Sonchus asper NC 048510.1	Tawang Mangu	905	905	100%	0	99.8%
Sonchus oleraceus MK371006.1	Tawang Mangu	905	905	100%	0	99.8%
Sonchus asper MF135322.1	Tawang Mangu	905	905	100%	0	99.8%
Embergeria grandifolia JQ933322.1	Tawang Mangu	905	905	100%	0	99.8%
Dendroseris marginate NC_051925.1	Tawang Mangu	905	905	100%	0	99.8%
Sonchus oleraceus NC 048452.1	Yogyakarta	905	905	99%	0	99.8%
Sonchus oleraceus MH908962.1	Yogyakarta	905	905	99%	0	99.8%
Dendroseris micrantha KY047631.1	Yogyakarta	905	905	99%	0	99.8%
Sonchus arvensis JX848427.1	Yogyakarta	905	905	99%	0	99.8%
Dendroseris macrantha NC_051924.1	Yogyakarta	905	905	99%	0	99.8%

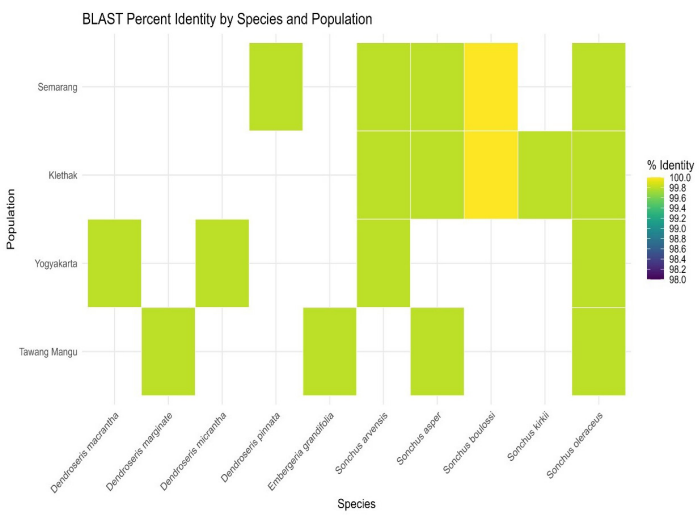

Figure 2. Heatmap of BLAST percent identity for *rbcL* gene sequences of *Sonchus* and related species across East Java populations.

related species like *S. masguindalii* and *S. palustris* (Table 3, Figure 3). Notably, the Yogyakarta population again displayed slightly lower percent identity (98.78–98.89%). Interestingly, BLAST results for both genes confirmed a strong genetic affinity with Eurasian *S. arvensis* lineages and not with native Australasian *Sonchus* species.

3.2. Phylogenetic reconstruction confirms taxonomic placement

Phylogenetic trees constructed from the *rbcL*.and *matK* alignments provided robust support for the taxonomic identity of the Javanese populations. The

Figure 3. Heatmap of BLAST percent identity for *matK* gene sequences of *Sonchus* and related species across East Java populations.

Table 3. BLAST results of *matK* Gene sequences from *Sonchus arvensis* ssp. *arvensis* populations in Java Island.

Species	Population	Max score	Total score	Query cover	E-value	Per. Ident
Sonchus arvensis DQ508001.1	Klethak	1738	1738	100%	0	99.89%
Sonchus masguindalii DQ508000.1	Klethak	1733	1733	100%	0	99.79%
Sonchus palustris DQ22985.1	Klethak	1733	1733	100%	0	99.79%
Sonchus boulosii NC 042244.1	Klethak	1727	1727	100%	0	99.68%
Prenanthes sp. GU109311.1	Klethak	1727	1727	100%	0	99.68%
Sonchus arvensis NC 054161.1	Semarang	1707	1707	100%	0	99.78%
Sonchus masguindalii DQ507998.1	Semarang	1707	1707	100%	0	99.78%
Sonchus palustris DQ022984.1	Semarang	1707	1707	100%	0	99.78%
Sonchus acaulis MK033507.1	Semarang	1701	1701	100%	0	99.68%
Sonchus aquatilis DQ507997.1	Semarang	1701	1701	100%	0	99.68%
Sonchus palustris DQ840446.1	Tawang Mangu	1698	1698	100%	0	99.89%
Sonchus fragilis DQ507992.1	Tawang Mangu	1698	1698	100%	0	99.89%
Sonchus acaulis NC 42382.1	Tawang Mangu	1692	1692	100%	0	99.78%
Sonchus canariensis MK033506.1	Tawang Mangu	1692	1692	100%	0	99.78%
Sonchus tenerrimus DQ507993.1	Tawang Mangu	1692	1692	100%	0	99.78%
Sonchus arvensis DQ508002.1	Yogyakarta	1696	1696	100%	0	98.89%
Sonchus fragilis DQ507991.1	Yogyakarta	1696	1696	100%	0	98.89%
Sonchus canariensis NC 042381.1	Yogyakarta	1690	1690	100%	0	98.78%
Sonchus boulosii MK016665.1	Yogyakarta	1690	1690	100%	0	98.78%
Sonchus luxurians DQ507986.1	Yogyakarta	1690	1690	100%	0	98.78%

Neighbor-Joining tree based on *rbcL* sequences placed all Javanese *S. arvensis* accessions within a clade containing reference sequences of *S. arvensis* and *S. boulosii* from Eurasia, supported by very short branch lengths indicative of minimal genetic divergence (Figure 4). The samples from Tawang Mangu, Yogyakarta, and Semarang formed a subclade, while the Klethak accession grouped directly with *S. boulosii* references. This clustering confirms the BLAST results and points to a shared, recent evolutionary history with these Eurasian taxa.

The phylogenetic tree reconstructed from *matK* sequences provided higher resolution and stronger support for these relationships (Figure 5). All Javanese accessions formed a single, well-supported monophyletic clade with reference sequences of *S. arvensis* from its native range (e.g., DQ508001.1). This clade was characterized by very short internal branch lengths. The *matK* tree also more clearly resolved the sister relationship between this *S. arvensis* clade and other closely related species like *S. fragilis* and *S. masguindalii*. As with the *rbcL* tree, native Australasian species and other genera (*Dendroseris, Embergeria*) were placed in distinct, well-separated clades.

Importantly, both trees confirmed that the Javanese accessions were phylogenetically distinct from the clade containing native Australasian species. Furthermore, the invasive *S. oleraceus* and *S. asper* formed a well-supported, separate clade in both analyses, highlighting the utility of these markers for distinguishing these common invasive congeners in Java. The overall high sequence conservation and the limited genetic variation observed among the four geographically separated Javanese populations for both *rbcL* and *matK* markers are reported here. Due to the low variation observed in these highly conserved chloroplast regions, conclusions about the number of introduction events or the exact source population cannot be drawn from these data alone.

4. Discussion

This study employed DNA barcoding with the chloroplast genes *rbcL* and *matK* to confirm the taxonomic identity of *Sonchus arvensis* subsp. *arvensis* in Java**.** Our analysis yields two principal findings: (1) the

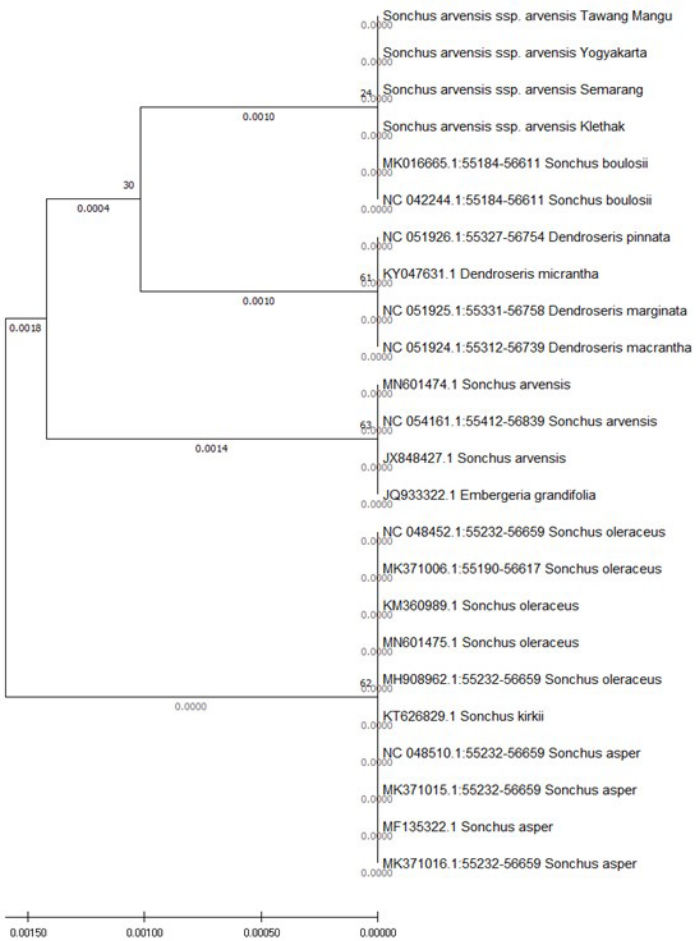

Figure 4. Neighbor-joining phylogenetic tree of *Sonchus* and related taxa based on *rbcL* sequences. Bootstrap values (1000 replicates) are shown at nodes. The scale bar indicates nucleotide substitutions per site.

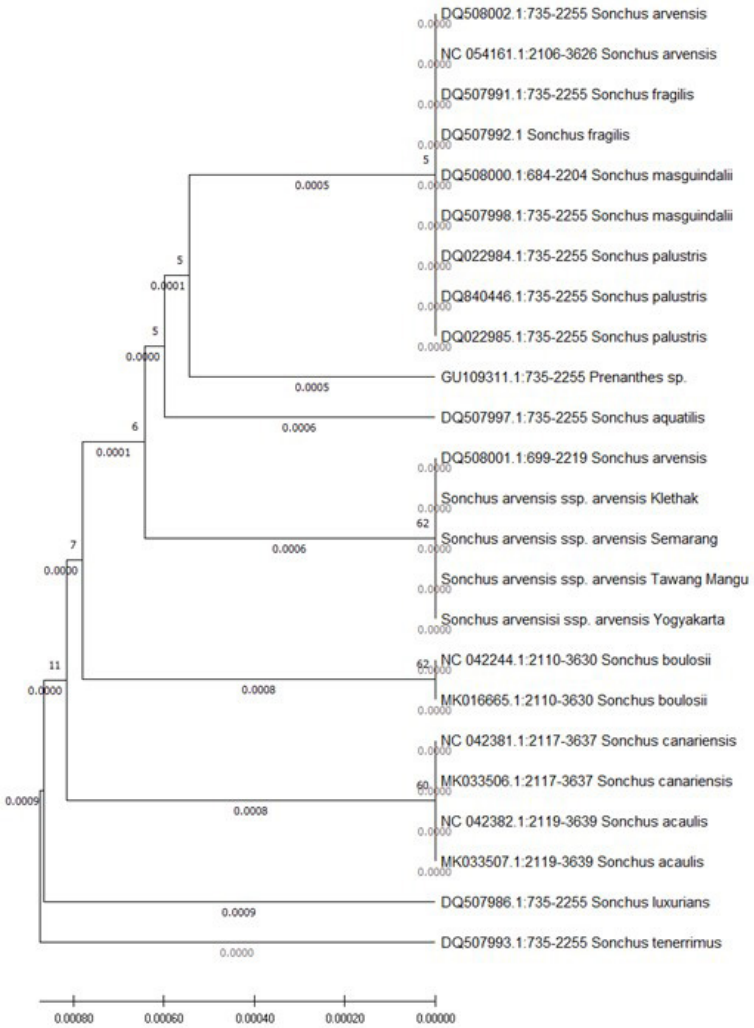

Figure 5. Neighbor-joining phylogenetic tree of *Sonchus* and related taxa based on *matK* sequences. Bootstrap values (1000 replicates) are shown at nodes. The scale bar indicates nucleotide substitutions per site.

successful identification of the Javanese populations as *S. arvensis*, and (2) their clear phylogenetic distinction from native Australasian *Sonchus* species. The primary result of this study is that chloroplast DNA barcodes *rbcL* and *matK* confirm that the sampled individuals belong to *S. arvensis* subsp. *arvensis*. This provides reliable species-level identification, which is crucial given the morphological ambiguities within the genus. While BLAST analysis revealed high sequence identity with several *Sonchus* species in global databases (including *S. boulosii*), phylogenetic reconstruction confidently placed our Javanese accessions within the *S. arvensis* complex. This confirms the initial morphological identification and is critical for accurately assessing the plant›s presence and distribution in Java.

Most importantly for conservation, our analysis demonstrates a clear phylogenetic separation between the invasive *S. arvensis* and the clade containing native Javanese and Australasian species. This genetic distinctness confirms that the Javanese accession is not a native species that has become weedy, but a true introduction.

The present study relied on the standard plant DNA barcode markers *rbcL* and *matK*. While these chloroplast regions are highly effective for species-level identification, they are also highly conserved and provide little resolution for intraspecific diversity (Hollingsworth et al., 2009; Letsiou et al., 2024). Consequently, our finding of limited sequence variation across Java should not be interpreted as evidence of a true lack of diversity or a single introduction event. Chloroplast markers are inherited as a single unit and do not capture nuclear diversity or fine-scale population structure. Nuclear markers (e.g., microsatellites, SNPs) would be required to robustly investigate population structure, founder effects, or local adaptation.

Furthermore, this study does not provide direct evidence regarding ecological interactions with native species, such as competitive exclusion or hybridization risks. The phylogenetic distinctness observed in chloroplast DNA

does not preclude the potential for hybridization, as this is governed by nuclear genome compatibility. Future studies incorporating nuclear markers and detailed field observations are needed to assess the potential for hybridization and the mechanisms of competition.

The high sequence conservation observed between *S. arvensis* and *S. boulosii*, while a challenge for resolving fine-scale taxonomy, is itself an insightful finding. It aligns with studies of Macaronesian *Sonchus*, which also show minimal plastid differentiation attributed to incomplete lineage sorting or ancient hybridization (Lee et al., 2005; Criado-Ruiz et al., 2024). The marginally lower sequence identity observed in the Yogyakarta samples could represent minor intraspecific variation. However, the limited variation in these chloroplast markers precludes definitive conclusions, highlighting the need for more variable genetic regions to detect such subtle population-level effects.

5. Conclusion

This work provides the first molecular confirmation of *S. arvensis* in Java using DNA barcodes. While it establishes species identity, further genomic studies are required to resolve the population history, introduction pathway, and ecological impact of this invasive species.

Acknowledgements

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Data Availability Statement

The entire dataset supporting the results of this study was published in the article itself.

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