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REVIEW ARTICLE

Molecular characterization for identifying the evolutionary lineage of Indonesia strawberry (*Fragaria* spp.) using DNA barcode markers: A review and current research on breeding strategies

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Abstract

Fragaria spp. (Strawberry) is one of the most popular plant species, with a high economic and nutritional value. It also plays a key role in evolutionary biology, particularly due to its widespread cultivation in Indonesia, where diverse cultivars are imported from abroad. Understanding the evolutionary paths of these cultivars is essential for grasping their genetic diversity. This review aims to explore the evolutionary dynamics of strawberry species found in Indonesia using a DNA barcoding approach. DNA barcoding is a powerful tool for uncovering the complex evolutionary histories of species and revealing their phylogenetic relationships. In this comprehensive review, we discuss various cultivated strawberry species present in Indonesia and examine the evolutionary forces that have driven their diversification. By analyzing genetic evidence, we seek a deeper understanding of the evolutionary processes behind these cultivars. However, challenges remain, particularly for *Fragaria* species, which face increasingly threats from the introduction and spread of "alien species". Addressing these challenges requires a holistic approach, that combines evolutionary biology principles with conservation strategies to protect native biodiversity while maximizing agricultural productivity. By enhancing our understanding of the evolutionary patterns and genetic evidence associated with cultivated strawberries in Indonesia, this review not only advances scientific knowledge but also provides a comparative phylogenetic tree of strawberry species in Indonesia. This serves as a foundation for informed conservation and breeding efforts in the constantly changing agricultural landscape.

Keywords: DNA barcoding; *Fragaria* spp.; molecular genetics; strawberry

Introduction

Strawberries (*Fragaria* spp.) are plants that produce fruits with high economic value and nutritional content (1). According to data from the Central Statistics Agency (2023), strawberry production in Indonesia reached 28895 t in 2022, marking a significant increase compared to the 9860 t produced in 2021 (2). The successful cultivation of strawberries in Indonesia is the result of cultivation from several countries, with 23 species and cultivars currently grown in the highlands of Indonesia (3).

Indonesia, despite being a subtropical country, has a high potential for the development of fruit commodities, including strawberries. Strawberry cultivation in Indonesia is primarily limited to highland areas, following the natural habitat of strawberries, which thrive in cold regions (1, 4) . The required temperature for strawberries to grow ranges from 20-25 °C. However, recent climate changes have increased air temperatures, necessitating strawberry adaptation (5). The adaptation of strawberries in Indonesia enables them to thrive at higher temperatures than those of their native habitat (6).

The cultivated strawberries in Indonesia are cultivars from abroad, but there is currently no research discussing the evolutionary forces that naturally occur in strawberries in Indonesia. Cultivated strawberries tend to undergo evolution due to human breeding. Previous study illustrates that a crossbreeding programme between *Fragaria* × *ananassa* cultivars from California and Hoybride, resulting in a new cultivar exhibiting resistance to fruit rot, high productivity and a sweet fruit flavour (7).

Generally, the relationship between genotype and phenotype reflects how genetic information is inherited from DNA and chromosomes and how this information produces observable physical characteristics in an individual (8). The interaction between genotype and phenotype in strawberries

can affect heritability and the relationship between the quality and quantity of its traits (8). Therefore, the outcomes of hybrid strawberries and new strawberry species can have different genotypes, resulting in phenotypic variations.

This research aims to understand and identify the evolutionary relationships among various strawberry species worldwide, especially in Indonesia, using DNA barcoding techniques.

Biodiversity of strawberry in Indonesia

The genus *Fragaria*, comprising approximately 23 species worldwide (Table 1), exhibits the same basic life history: they are insect-pollinated, low-growing, herbaceous perennials capable of clonal propagation and produce animal-dispersed fleshy accessory fruits (9). However it can also have fascinating diversity in both ploidy levels and morphological traits. Most commonly, species such as wild strawberry *Fragaria vesca* are diploid (2n = 14), while others, like *Fragaria* × *ananassa* (garden strawberry), are octoploid (2n = 56) (10). This variation in ploidy significantly influences their morphological characteristics, including leaf shape, flower size and fruit characteristics. Typically, diploid species bear smaller, more delicate flowers and fruits, while polyploidy species tends to produce larger, more robust fruits with increased sweetness and varying colors (9).

The floral and vegetative morphology of *Fragaria* species is relatively uniform. Leaves are usually evergreen (with *F. iinumae* being an exception as it is deciduous) and trifolioliate. While 5 leaflets are common among some Chinese species, the occurrence of 4-5 leaflets is rare and has been observed in *F. virginiana* and *F. cascadensis* (11). The flowers are always actinomorphic, predominantly white (sometimes tinged with pink) and usually possess 5 petals (9).

Indonesia boasts high biodiversity, also hosts a variety of strawberry cultivars. The country currently lacks a National Biodiversity Index (IBI) to monitor biodiversity trends comprehensively. As such, there is a pressing need to prioritize conservation, identification and systematic documentation, particularly in relation to their potential inclusion in the Indonesian Biodiversity Index (12). This effort should encompass cultivars, hybrids and native species. There are several compelling reasons to support the conservation and identification of strawberry species in Indonesia, including the preservation of biodiversity, improved agricultural productivity, enhanced fruit quality and increased economic value of strawberries at the national level (13). Taking proactive steps to conserve and identify strawberries in accordance with the IBI will contribute both to biodiversity conservation and to the economic and agricultural development of the country (12, 14) .

Based on Table 2, the majority of identified strawberries in Indonesia belong to the hybrid species *F* × *ananassa*, which has been imported for food production purposes (3). These hybrid species have been distributed in several locations, namely West Java, Central Java, East Java, North Sumatra and Bali (3, 7, 16 -18). The distribution of these cultivars is closely linked to environmental suitability, as strawberries generally

Table 1. *Fragaria* species and their ploidy level, mating system and geographic distribution (9)

Taxon	Ploidy	Mating system	Geographic distribution
Vesca clade			
F . × *ananassa* Duchesne subsp. *ananassa*	8x	Subdioecious (modern cultivars: hermaphroditic)	Cultivated around the world
F . × *ananassa* Duchesne subsp. *cuneifolia* (Nutt. ex Howell) Staudt	8x	Subdioecious	NW N. America
F. bucharica Losinsk.	2x	Hermaphrodite SI	W Himalayas
F. cascadensis Hummer	10x	Subdioecious	Oregon, USA
F. chiloensis (L.) Duchesne	8x	Subdioecious	Alaska-California; Hawaii; Chile, Argentina
F. iturupensis Staudt	8x, 10x	Subdioecious	Iturup Island
F. mandshurica Staudt	2x	Hermaphrodite SI	NF Asua
F. moschata Duchesne	6x	Dioecious	W Eurasia
F. orientalis Losinsk.	4x	Dioecious	NE Asia
F. vesca L. subsp. *americana* (Porter) Staudt	2x	Hermaphrodite SC	NE N. America
F. vesca L. subsp. *bracteata* (A. Heller) Staudt	2x	Gynodioecious or Hermaphrodite SC	W N. America
F. vesca L. subsp. *californica* (Cham. & Schldl.) Staudt	2x	Gynodioecious or Hermaphrodite SC	SW N. America
F. vesca L. subsp. *vesca* L.	2x	Hermaphrodite SC	W Eurasia
F. virginiana Duchesne	8x	Subdioecious	N. America
China clade			
F. chinensis Losinsk.	2x	Hermaphrodite SI	China
F. corymbosa Losinsk.	4x	Dioecious	China
F. daltoniana J. Gay	2x	Hermaphrodite SC	Nepal, adjacent China
F. gracilis Losinsk.	4x	Dioecious	China
F. moupinensis (Franch.) Cardot	4x	Dioecious	China
F. nipponica Makino	2x	Hermaphrodite SI	Japan
F. nubicola Lindl.	2x	Hermaphrodite SI	Himalayas
F. pentaphylla Losinsk	2x	Hermaphrodite SI	China
F. tibetica Staudt & Dickore	4x	Dioecious	China
Unresolved phylogenetic position			
F. hayatai Makino	2x	Hermaphrodite SC?	Taiwan
F. iinumae Makino	2x	Hermaphrodite SC	Japan
F. nilgerrensis Schltdl. ex J. Gay	2x	Hermaphrodite SC	SE Asia
F. viridis Duchesne	2x	Hermaphrodite SI	W Eurasia

Table 2. Strawberry species cultivated in Indonesia

No	Cultivars	Locations	Ref.
1	*Fragaria* × *ananassa* cv. Camarosa	-	
2	*Fragaria* × *ananassa* cv. Sunset	-	
3	*Fragaria* × *ananassa* cv. Selva	Purbalingga, Karanganyar, Magelang (Center Java)	
4	*Fragaria* × *ananassa* cv. Carmine	-	
5	*Fragaria* × *ananassa* cv. Pajero	-	
6	*Fragaria* × *ananassa* cv. Sweetcharlie	Ciwidey, Garut, Lembang (Center Java) & Batu, Pasuruan, Bondowoso, Magetan (East Java) & Bedugul (Bali) & Kecamatan Berastagi (North Sumatera)	
7	*Fragaria* × *ananassa* cv. Festival	-	
8	*Fragaria* × *ananassa* cv. Earlybrite	Ciwidey, Garut, Lembang (West Java)	
9	*Fragaria* × *ananassa* cv. Cal Giant	-	
10	*Fragaria* × *ananassa* cv. Aromas	-	
11	*Fragaria* × *ananassa* cv. Diamente	-	
12	*Fragaria* × *ananassa* cv. Giavonta	-	
13	*Fragaria* × *ananassa* cv. Terry	-	
14	*Fragaria* × *ananassa* cv. Adina	-	
15	*Fragaria* × *ananassa* cv. Collma	-	(3)
16	*Fragaria* × *ananassa* cv. Whitney	-	
17	*Fragaria* × *ananassa* cv. Chandler	Pasuruan, Bondowoso, Magetan (East Java)	
18	*Fragaria* × *ananassa* cv. Ozogrande	Purbalingga, Karanganyar, Magelang (Center Java) & Kecamatan Berastagi (North Sumatera)	
19	*Fragaria* × *ananassa* cv. Rosalinda	Batu (East Java) & Bedugul (Bali)	
20	*Fragaria* × *ananassa* cv. Kanaka Peak 794-15	-	
21	*Fragaria* × *ananassa* cv. Kanaka Peak 770-506	-	
22	*Fragaria* × *ananassa* cv. Winter down	-	
23	*Fragaria* × *ananassa* cv. Icigo	-	
24	*Fragaria* × *ananassa* cv. California	Ciwidey, Garut, Lembang (West Java)	
25	*Fragaria* × *ananassa* cv. Holland	Ciwidey, Garut, Lembang (West Java) & Pasuruan, Bondowoso, Magetan (East Java)	
26	*Fragaria* × *ananassa* cv. Anna	Purbalingga, Karanganyar, Magelang (Center Java)	
27	*Fragaria* × *ananassa* cv. Tristar	Purbalingga, Karanganyar, Magelang (Center Java)	
28	*Fragaria* × *ananassa* cv. Quantum	Purbalingga, Karanganyar, Magelang (Center Java)	
29	*Fragaria* × *ananassa* cv. Berastagi	Berastagi (North Sumatera)	
30	*Fragaria* × *ananassa* cv. Dorit	Berastagi (North Sumatera)	
31	*Fragaria* × *ananassa* cv. Hoybride	Batu (East Java)	(7)
32	*Fragaria* × *ananassa* cv. Santung	Malang (East Java)	(15)
33	*Fragaria* × *ananassa* cv. Aerut	Malang (East Java)	
34	*Fragaria* sp.	Pekanbaru (Riau)	(16)
35	*Fragaria* sp.	Buleleng (Bali)	(17)

Fig. 1. Types of growth of nine cultivars of strawberries. (A) Californica, (B) Rosa Linda, (C) Berastagi, (D) Festival, (E) Santung, (F) Holland, (G) Sweet Charlie, (H) Aerut, (I) Earlibrite (Upright: A and H; Intermediate: B-G and I) (15).

thrive in highland areas at elevations of around 1000 m above sea level (3).

Fragaria × *ananassa* originated from a natural hybridization event between *Fragaria chiloensis* and *Fragaria virginiana*, which occurred in Europe during the 18th century. This hybridization resulted in the mixing of genetic material from both species, yielding new offspring with unique morphological characteristics and genetic sequences (9). Over time, various cultivars of *F.* × *ananassa* have been developed through selective breeding to enhance desired traits such as fruit size, flavor, yield, disease resistance and adaptability to different growth conditions. The breeding process involves crossing various cultivars and selecting individuals with favourable traits for further propagation (18). These hybrid cultivars often exhibit morphological and physiological adaptations that contributes to increase disease resistance and faster growth (19).

Several cultivars of strawberries found in Indonesia, namely Californica, Rosa Linda, Berastagi, Festival, Santung, Holland, Sweet Charlie, Aerut and Earlibrite, exhibit differences in morphological characteristics. Among these cultivars, Sweet Charlie and Aerut have an upright growth

type, whereas Californica, Rosa Linda, Berastagi, Festival, Santung, Holland and Earlibrite have an intermediate growth type. Furthermore, concerning fruit shape, 4 dominant types are observed: ovoid (Californica, Santung and Holland); cylindrical, (Sweet Charlie); conical (Rosa Linda, Berastagi and Festival) and cordate (Aerut and Early Brite) (Fig. 2)(15).

The evolutionary history of the wild genus *Fragaria* is distinguished by complex interactions with genetic diversity, hybridization, varying ploidy levels and wide geographic distribution. Based on Fig. 3, the genus *Fragaria* comprises a number of species exhibiting diverse ploidy levels, including diploid (2x), tetraploid (4x), hexaploid (6x) and octaploid (8x). The cultivated strawberry, *F.* × *ananassa*, is the result of a hybridization event between the wild species *F. virginiana*, native to North America and *Frchiloensis*, from South America. This hybridization led to the development of a novel species that has become the dominant strawberry crop globally. Notably, the diploid species *F. vesca* served as a primary root in the establishment of this evolutionary lineage. Recent genomic studies have further elucidated the evolutionary dynamics within *Fragaria*, revealing multiple diploid progenitor species and highlighting the influence of environmental factors in shaping genetic diversity (20, 21) .

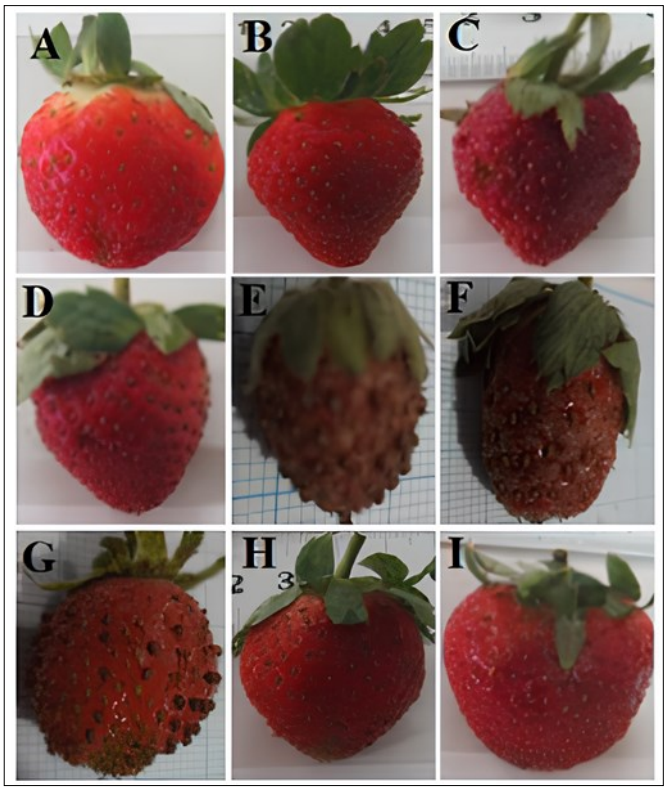

Fig. 2. Fruit shapes of nine cultivars of strawberries: ovoid (A, E, F), cylindrical (G), conical (B, C, D) and cordate (H, I). The cultivars are (A) Californica, (B) Rosa Linda, (C) Berastagi, (D) Festival, (E) Santung, (F) Holland, (G) Sweet Charlie, (H) Aerut and (I) Earlibrite (15).

Based on Fig. 4, *F.* species selected for analysis were collected from diverse regions, including India, China, Japan, East Asia, Southeast Asia, America, Europe and the Kuril Islands. Phylogenetic analysis of *Fragaria* cultivars and species worldwide indicates that *F.* × *ananassa* is most closely related to *F. virginiana.* This relationship exists because *F. ananassa* is a hybrid of *F. virginiana* and *F. chiloensis* (22, 23). This phylogenetic analysis is further supported in Fig. 5, which examines the phylogeny of *Fragaria* found in Pakistan and highlights the crucial role of gene inheritance in *Fragaria* evolution.

The incorporation of outgroups in phylogenetic analysis is crucial, as it establishes a baseline for comparison, clarifies evolutionary relationships, minimizes ambiguity and enhances analytical precision. In this study, *Potentilla fruticosa* was selected as an outgroup based on its morphological and ecological similarities to *Fragaria* species (25). Moreover, the distribution of these 2 plants overlaps, making them suitable for evolutionary comparison. This is supported by a previous study, which revealed *Fragaria*'s close relationship with the *Potentilla* clade, one of which includes *P. fruticosa* (22).

Essentially, species are defined as individual entities or groups sharing consistent characteristics. Meanwhile, cultivars are varieties of plant species that have been selected or bred for specific characteristics and cultivated to exhibit specific traits (26). In other words, species are groups of organisms with similar characteristics, while cultivars are

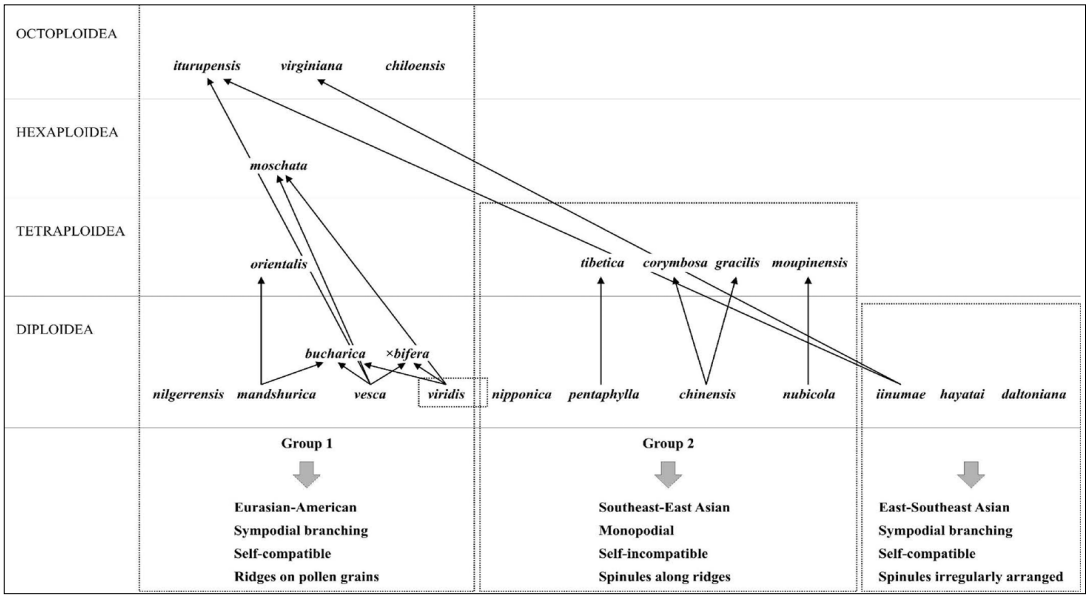

Fig. 3. Phylogenetic analyses of wild type strawberry based on number of chromosomes (20).

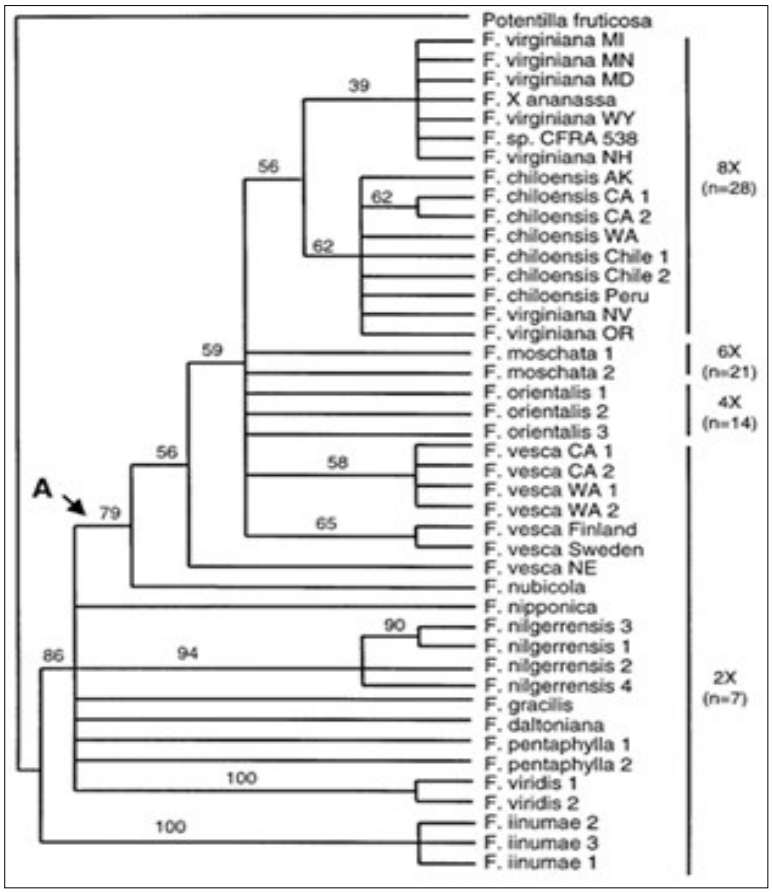

Fig. 4. Phylogenetic analysis of *Fragaria* based on maximum parsimony worldwide (22).

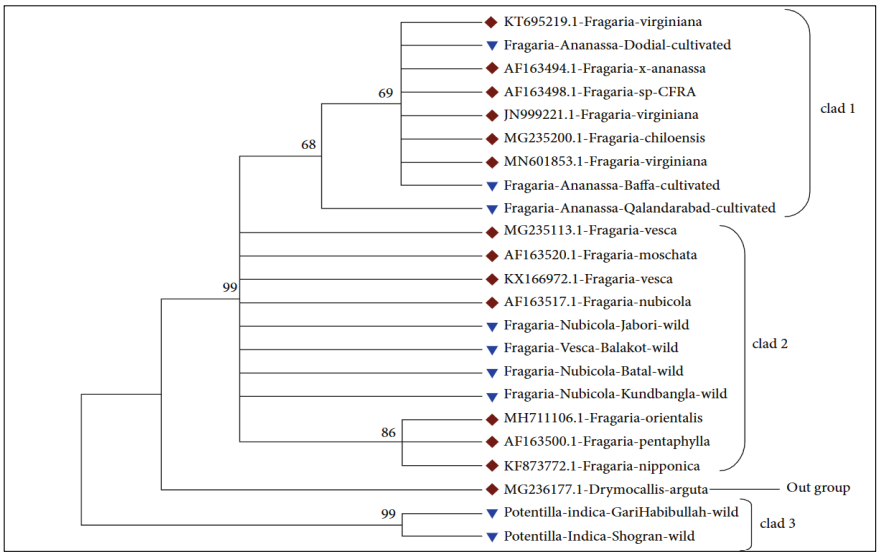

Fig. 5. Phylogenetic analysis of *Fragaria* based on ITS2 gene by maximum Likelihood in Pakistan (27).

varieties resulting from selective breeding to obtain specific traits.

However, due to the government's limited attention to the biodiversity of strawberry plants in Indonesia, there is an urgent need for a conservation approach to protect, document and collect them using DNA barcoding. DNA barcoding is an essential tool in the conservation context, as it enables rapid and accurate species identification, aiding in the recognition of various species, whether invasive or endangered and supporting their management and protection. Additionally, DNA barcoding enables the measurement of phylogenetic diversity, offering a more detailed and descriptive picture of biodiversity compared to alternative indices such as species richness and abundance (28).

Genetic conservation aims to preserve the genetic material of organisms to minimize the risk of extinction and the threat of population decline that may occur through genetic and evolutionary processes (29, 30). Thus, DNA barcoding is crucial in estimating species richness and establishing conservation priorities, especially in areas with minimal information about their biodiversity. Moreover, this method can help identify small areas that are important for conservation efforts, considering locations that have served as natural refuges for certain species in the past and areas that have recently become home to new lineages (28). Conservation efforts through genetic approaches have been undertaken in several plants, especially strawberries, such as

Fragaria spp. (31) and wild *Fragaria* (32).

The analysis of the phylogenetic tree of *Fragaria* species was conducted using three genes, including ITS2, *rbc*L and *mat*K, employing both Maximum Likelihood (ML) and Bayesian Inference (BI) methods (Fig. 6-8). In general, the results from both methods exhibited high similarity; therefore, only the phylogenetic trees constructed using the ML method are presented, supported by statistical values from both ML and BI analyses.

Based on Fig. 6, the results of the phylogenetic analysis of *Fragaria* species concerning the ITS2 gene can be observed using ML and BI methods. These results show similarities to previous studies (22) (Fig. 4). Overall, the closest relationship is between *F virginiana, F* × *ananassa* and *F chiloensis*. Additionally, among the three outgroups used, the results suggest that *Fragaria* species are more closely related to *Spyridium buxifolium* and *Stenanthemum centrale* than *Spirae humilis*.

Fig. 7 presents the results of the phylogenetic analysis based on the *rbc*L gene utilizing both ML and BI methods. The results indicate that the most *Fragaria* species share a similar evolutionary relationship. However, *F. nilgerrensis* is situated in a distinct lineage compared to other *Fragaria* species. Additionally, among the 3 outgroups, *Rosa multiflora* and *Potentilla angelica* exhibit a closer relationship than *Geum henryi*.

Similarly, Fig. 8 illustrates the results of the phylogenetic analysis of *Fragaria* species using the *mat*K gene with ML and BI methods. These results exhibit similarities to the phylogenetic analysis results of *Fragaria* species with the *rbc*L gene (Fig. 7). The analysis demonstrates that various *Fragaria* species, such as *F. virginiana, Fragaria* × *ananassa, Fragaria vesca, Fragaria orientalis, F. chiloensis* and *F. sp.,* are in the same lineage. However, *F. nilgerrensis* once again forms a distinct branch from the other *F.* species. Additionally, among the 3 outgroups utilized, the results suggest that *Fragaria* species have a closer relationship to *Potentilla*

Fig. 6. Phylogenetic analysis *Fragaria* using ITS2 gene based on maximum-likelihood (ML) and Bayesian Inference (BI).

Fig. 7. Phylogenetic analysis *Fragaria* using *rbc*L gene based on Maximum-Likelihood (ML) and Bayesian Inference (BI).

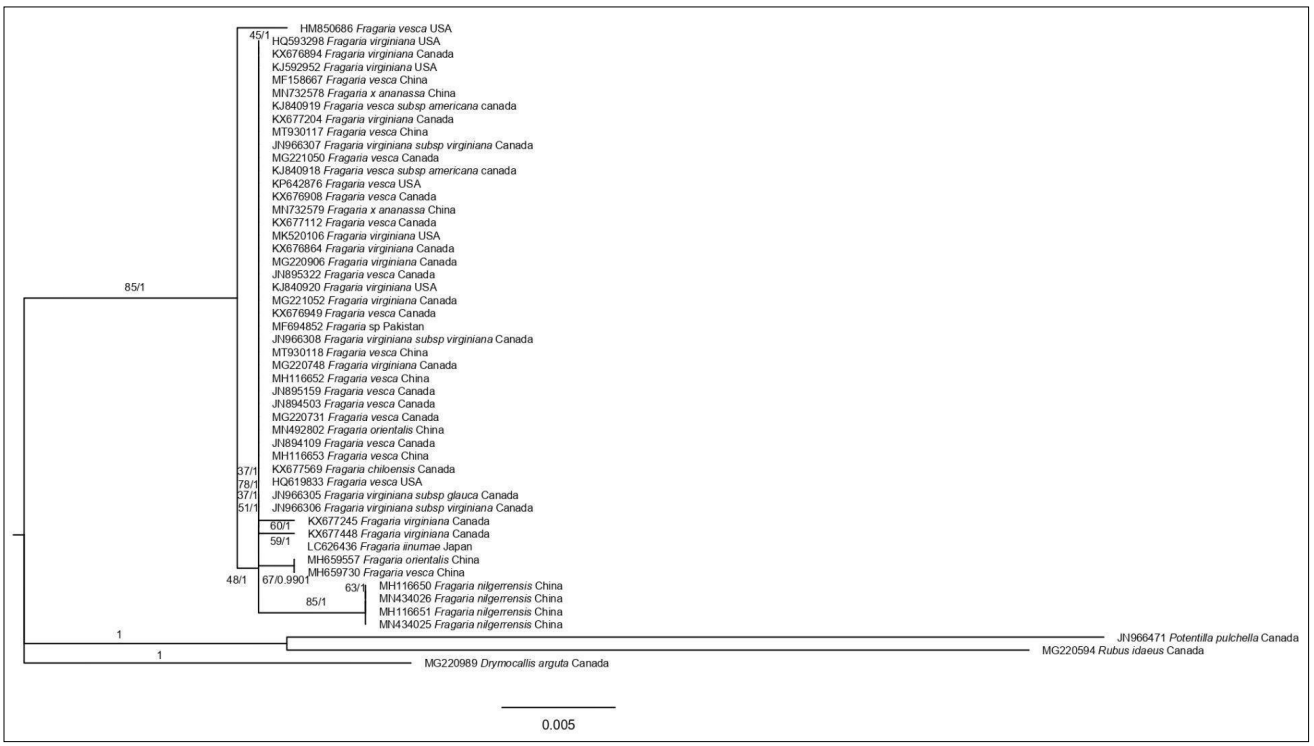

Fig. 8. Phylogenetic analysis *Fragaria* using *mat*K gene based on Maximum-Likelihood (ML) and Bayesian Inference (BI).

pulchella and *Rubus idaeus* than *Drymocallis arguta*.

Correlation between molecular genetics analysis and molecular systematics

Molecular genetics is one of the latest technologies that can be used to reveal the identity of a new species by leveraging the genetic information of an organism and comparing its DNA or RNA sequences with those of known organisms (33). Strawberries, which belong to the Rosaceae family, have undergone significant variations in taxonomic classification over the last few decades, with various classification methods proposed (34). The complexity of taxonomy and the limitations of morphological characteristics within this family pose difficulties in identifying species based solely on morphological taxonomy. Therefore, Rosaceae is an ideal subject for identification using DNA barcoding methods, as this family presents taxonomic barriers that complicate the separation of closely related species within the family using

conventional approaches (35).

DNA barcoding is a highly useful method for taxonomic identification, utilizing short, universally conserved DNA segments that have sufficient sequence variations to distinguish species (36, 37). The primary goal of the DNA barcoding approach is to authenticate known species by matching their DNA sequences with references in the barcode sequence library and to facilitate the recognition of new species (36, 38). For example, the mitochondrial cytochrome c oxidase 1 (cox1) gene sequence has been used as a universal barcode in identifying several groups of animals (39). Meanwhile, the combination of the *mat*K + *rbc*L genes has been proposed as the nuclear DNA barcode for plant identification by the Consortium for the Barcode of Life (CBOL) Plant Working Group (PWG), which also suggests using ITS2 (internal transcribed spacer 2) as an additional identifier (35). the study DNA barcodes such as ITS, ITS2, *mat*K, *rbc*L, *rbc*LA, *rbc*LC, *Ycf*3 and *trn*V were used to characterize strawberries from Mansehra, Pakistan and determine their relationships and phylogenetic tree (33).

The % of similarity between species, derived from genetic analysis, provides insights into their evolutionary history by revealing how closely related they are. A high % of similarity indicates that 2 species share a more recent common ancestor, reflecting a closer evolutionary relationship. Conversely, a lower % suggests a more distant evolutionary connection (40). Based on Table 3 the % of similarity between the *Fragaria* species indicates a high % similarity suggesting that these species share a more recent common ancestor, indicating closer evolutionary ties. Based on Table 3, the percentage of similarity among *Fragaria* species indicates a high % of similarity, suggesting that these species share a recent common ancestor, indicating closer evolutionary ties. Specifically, *F. chiloensis, F. virginiana* and *F.* × *ananassa* in particular exhibit 99.9% to 100% similarity. This finding is consistent with their evolutionary history, as *F.* × *ananassa* is a cross between *F. chiloensis* and *F. virginiana* (22).

Before molecular genetics was applied as a species identification technique, several other identification techniques existed, such as morphology, anatomy, physiology and biochemistry-based identification. Each of these identification methods has its advantages and disadvantages. The advantage of molecular taxonomy is its ability to identify species that are difficult to identify by other means, such as species with similar morphologies or species that are challenging to differentiate physiologically or biochemically (33). Another major advantage lies in the fact that DNA sequences are directly inherited and are not subject to developmental or environmental influences that may obscure morphological traits. Consequently, molecular taxonomy provides a more stable and objective basis for species identification. Moreover, it allows for the identification of future evolutionary lineages based on their genetic sequences (33).

However, molecular taxonomy is not without its limitations. Its application still relies on species definitions provided by morphological taxonomy, making the 2 methods interdependent. Additionally, the accuracy of molecular identification may be compromised by potential contamination, which can distort the results of genetic analyses (41).

Table 3. Percent similarity of *Fragaria species* using DNA barcoding

Taxon	Percent Identity (%)	Accession
Fragaria × *ananassa*	100	OP723290
Fragaria virginiana	99.99	NC_019602
Fragaria moupinensis	99.41	MZ702805
Fragaria tibetica	99.41	MZ702810
Fragaria gracilis	99.41	NC_062837
Fragaria chiloensis	100	MW537844
Fragaria tibetica	99.41	NC_062835
Fragaria vesca subsp. *vesca*	99.77	MZ851769
Fragaria mandshurica	99.80	MZ851758
Fragaria pentaphylla	99.51	NC_034347
Fragaria chinensis	99.51	MZ702808
Fragaria nubicola	99.49	MW537841
Fragaria corymbosa	99.48	MZ851751
Fragaria nilgerrensis	99.49	MZ702804
Fragaria nipponica	99.47	MW537843.
Fragaria daltoniana	99.39	MZ851755
Fragaria iinumae	99.39	MW537854
Fragaria orientalis	99.72	MW537853

The development of DNA barcoding as an accurate identification tool

DNA barcoding is used because it can identify species quickly and inexpensively (42). It is estimated that morphological analysis incurs higher costs and takes several months of fieldwork compared to species sequencing (43). However, there are challenges in applying DNA barcoding to plants because the genes used as phylogenetic markers have very limited variations, making them insufficient to differentiate species. Therefore, using techniques based on a chloroplast region is highly recommended (44).

Several genes commonly used for DNA barcoding include *rbc*L, *mat*K, *cox*1 and 16S (36, 45). The selection of appropriate genes is based on several factors: the marker should exhibit sufficient genetic variability and divergence at the species level, possess conserved regions suitable for the development of universal PCR primers for taxonomic applications and have a relatively short sequence length to facilitate efficient DNA extraction and amplification (46). Nonetheless, gene selection must be contextual, as certain genes are more suitable for specific taxonomic groups. For instance, the Internal Transcribed Spacer (ITS) region is commonly used for fungi because it is one of the most frequently sequenced markers and is routinely applied in systematics, phylogenetics and species identification (47). Therefore, gene selection should be guided by the research objectives and the biological characteristics of the species under investigation. The development of DNA barcoding for the identification of strawberry species involves the use of specific DNA sequences of genes, such as chloroplast DNA (Table. 4). Chloroplast partial (cp) has a genome that contains information for taxonomic and phylogenetic purposes, making it often used in plant identification research, including strawberries (31). In Fig. 9, *Potentilla*

Table 4. Function and characterization DNA barcode (8)

Genes	Genome	Organism	Functions	Characterization
*mat*K	Chloroplast	Plant	Intra and Interspecies Taxonomy	Small genome in size, middle in variability and middle in mutation
*rbc*L			Interspecies	Small genome in size, the mutation is lower than *mat*K, *ITS* and *rbc*L
ITS1-rDNA	Chromosomal		Intra and Interspecies Taxonomy	Degree of variation of genome is higher than *18S, mat*K *and rbc*L
ITS2-rDNA				
18S-rDNA				*Conserved* gene
COI-barcode	Mitochondria	Animals	Intraspecies	conserved, stable, fast evolutions

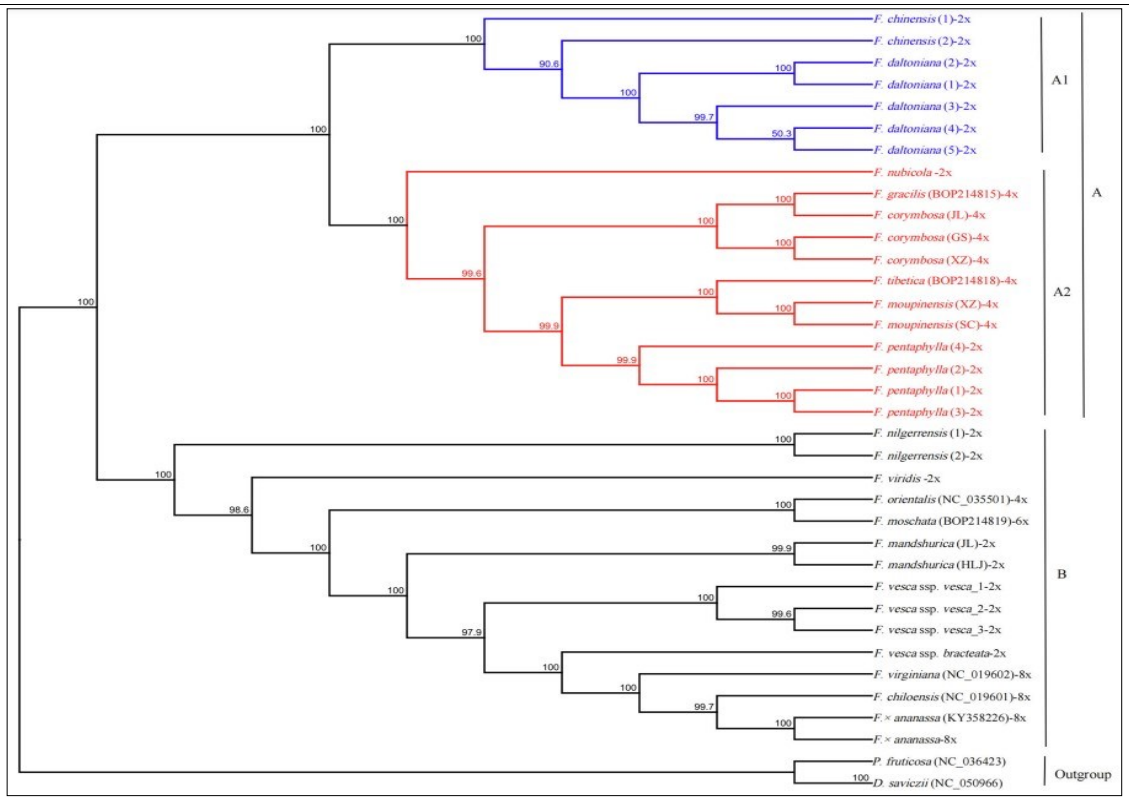

Fig. 9. Phylogenetic analysis of *Fragaria* based on partial chloroplast sequences (31).

fruticosa and *Drymocallis saviczii* are used as outgroups with partial chloroplast genome sequences from 34 *Fragaria* accessions. Additionally, there is research focused on molecular phylogenetic analysis in the *Fragaria* genus based on whole chloroplast genome sequences (32). In Fig. 10 the outgroups used include *Rosa multiflora, Potentilla stolonifera* and *Malus hupehensis*, with whole chloroplast genomes from 20 *Fragaria* samples.

Opportunities and challenges

Difficulties in identifying new *Fragaria* species in Indonesia are often encountered, particularly due to the presence of numerous alien species (48). These alien species may be introduced either intentionally or unintentionally (48, 49) . In Indonesia and other agronomically focused countries, such introductions often occur for cultivation experiments or through botanical garden collections (48). As a result, the presence of alien species reduces the genetic variation within *Fragaria*, frequently leading to the dominance of these introduced species (50). This situation is further exacerbated by limitations in DNA barcoding analysis, which complicates accurate identification (51). DNA may be degraded or lost during the analysis process, leading to partial or fragmented genetic data (52). Additionally, genetic sequences available in the National Center for Biotechnology Information (NCBI) database are often unpublished or lack sufficient detail, with some studies failing to specify the genes associated with particular species (52). As a result, *Fragaria* identification becomes inaccurate or unsuccessful, as seen in a study, which failed to identify *Fragaria* species due to limited psbA-trnH genes and the use of other genes with limited chloroplast DNA (53).

The cultivation of alien species in close proximity to natural habitats also increases the potential for gene flow (54). In Central Europe, *Feral F.* × *ananassa* has emerged in areas near abandoned strawberry gardens. However, this species has not yet been recorded in the NCBI GenBank database (55). While such occurrences may pose a risk to native species, the impact remains limited due to the rarity of hybridization events and the presence of reproductive barriers that restrict gene flow between cultivated and wild species (49, 55). It is still unclear whether pollinators significantly contribute to natural hybridization (55). Nonetheless, the possibility of hybridization affecting *Fragaria* diversity in Indonesia highlights the need for further research into species identification.

Beyond the challenge of distinguishing alien from native species, the genetic complexity of F. also presents difficulties due to its monogenic and polygenic characteristics (57, 58). In polygenic species, DNA barcoding is a time-intensive process, requiring the discrimination of multiple barcode

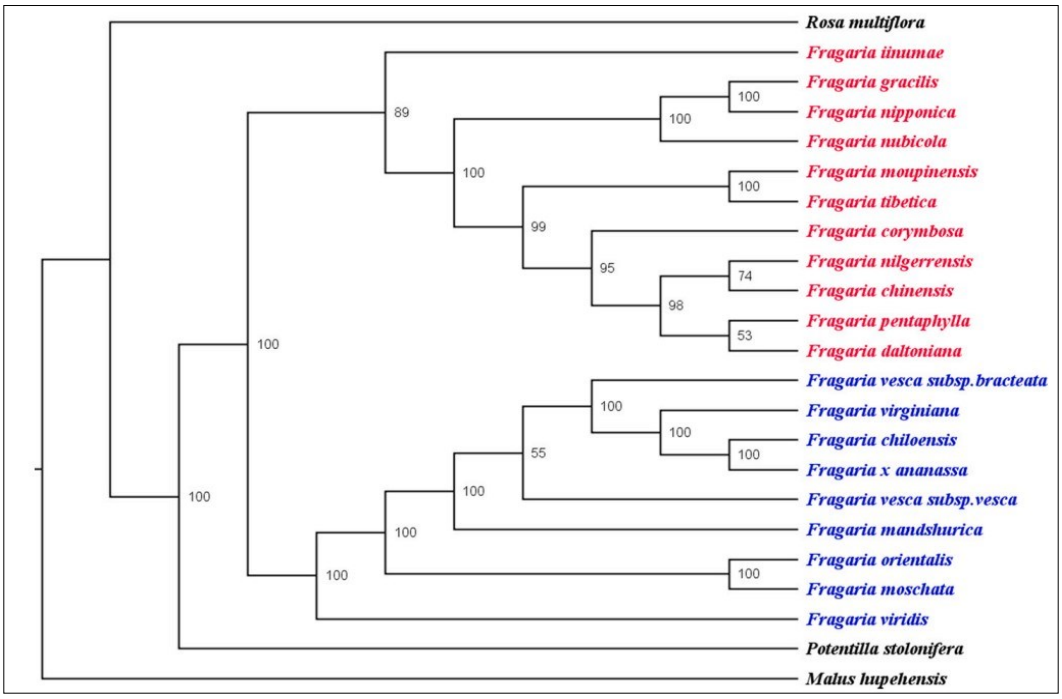

Fig. 10. Phylogenetic analysis of *Fragaria* based on full-length chloroplast sequences (47).

genes (61). Depending on the species in question, certain genes, such as matK, may provide a more accurate reflection of plant evolutionary history than ITS2 (58). However, DNA barcoding is limited in its ability to account for environmental influences on polygenic traits (58, 59). Similarly, challenges exist for monogenic species, where successful identification depends on the availability of the exact target gene for sequencing. Unfortunately, the limited reference data for *Fragaria* in NCBI makes it difficult to obtain precise identification (Table 5)(52, 60).

Despite these limitations, DNA barcoding remains an effective method for species identification and for elucidating evolutionary histories (Table 6) (62). A previous study demonstrated that DNA barcoding analysis of *Fragaria* species can produce a phylogenetic tree with a bootstrap value exceeding 99% (31). Additionally, vegetation evolutionary analyses conducted on Barro Colorado Island and Ailao Mountain using *rbc*L, *mat*K and *trn*H-*psb*A genes have shown high reliability (62). These findings underscore the important role of DNA analysis in understanding species evolution and highlight the urgent need to conserve genetic diversity (63).

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Authors' contributions

GRA carried out validation data, supervision, drafted and finalization the manuscript. MAA carried out molecular genetic studies, taking data and articipated in the sequence alignment and drafted the manuscript. MF carried out molecular genetic studies molecular genetic studies, taking data and articipated in the sequence alignment and drafted the manuscript. MSB carried out molecular genetic studies molecular genetic studies, taking data and articipated in the sequence alignment and drafted the manuscript. RSK participated in the design of the study and performed the statistical analysis. AW conceived of the study and

Table 5. Characterization of genetic marker in plant DNA barcoding (56)

Marker	Type	Basa size	Number of accessions in genebank	Number of species in genebank
nrITS2	*Nuclear*	157–670	111370	57579
*mat*K	Plastid	862–910	34647	22701
*rbc*L	Plastid	654–654	27725	20374

Table 6. Challenges and opportunities of DNA barcoding

Challenges	Opportunities
Alien species	An increasing number of species for identification Identification of hybrid species
Monogenic and Polygenic	Research involving various other Barcoding genes
Limited information	Collaborative research with research institutions Opportunities for additional research (Research Gap)

participated in its design and coordination. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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