BIODIVERSITAS
Volume 24, Number 5, May 2023
Pages: 2938-2952

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240549

Genetic diversity of the genus *Citrus* in Tomini Bay coastal areas, Indonesia based on morphological characters

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Manuscript received: 16 November 2023. Revision accepted: 27 May 2023.

Abstract. *Kandowangko NY, Febriyanti. 2023. Genetic diversity of the genus* Citrus *in Tomini Bay coastal areas, Indonesia based on morphological characters. BIodiversitas 24: 2938-2952. Citrus* plants are one of the fruit plants that are widely consumed by the community, both for food, health, and customs. The existence of *Citrus* plants in the coastal area of Tomini Bay only grows wild and has not been cultivated intensively. Lack of information on the diversity of citrus and the use of *Citrus* plants that are not balanced with cultivation actions will threaten the existence of these *Citrus* plants. This research aims to provide information about the morphological characteristics of the genus *Citrus* in the coastal area of Tomini Bay. The plant materials used were 39 accessions consisting of 7 species. Descriptive methods and diversity index calculations as well as analysis of phenetic consanguinity data were applied in this study. The results showed that all *Citrus* accessions have morphological diversity ranging from stems, leaves, flowers, fruits, and seeds that are influenced by genetic and environmental factors. The diversity index (H') was equal to 0.8103 which was categorized as low. The 39 *Citrus* accessions were divided into two main clusters with a similarity index of 64.3%. The findings of this study are expected to be useful in *Citrus* breeding and conservation programs in the future.

Keywords: *Citrus*, genetic diversity, morphological characters, Tomini Bay

INTRODUCTION

Citrus (*Citrus* spp.) is Indonesia's second most popular fruit, after banana. Indonesia's total *Citrus* production in 2021 was 2,520,256 tons (Central Bureau of Statistics Indonesia 2022). *Citrus* contains several phytochemical components that are beneficial to health, including vitamin C, vitamin B, potassium, calcium, phosphorus, magnesium, manganese, salt, carotenoids, and other phenolic chemicals (Abobatta 2019). *Citrus* is also employed in many traditional ceremonies in Indonesia. *Citrus hystrix* DC., for example, is used for bathing the body in Tanjung Botung Village, North Sumatera (Rambey and Lubis 2022) and *Citrus x aurantiifolia* (Christm.) Swingle is utilized as a complimentary substance to send prayers in a traditional Minangkabau ritual, West Sumatra (Des et al. 2019). Based on data from the Central Bureau of Statistics Indonesia (2021), consumption of *Citrus* by the household sector in 2021 reached 1,153,430 tons, an increase of 29.95% (265,810 tons) from 2020.

Some species of *Citrus* are native to Indonesia, such as *Citrus grandis* (L.) Osbeck (Kalimutu et al. 2020), *Citrus amblycarpa* (Hassk.) Ochse (Budiarto et al. 2017), and *C. hystrix* (Boning 2021). Recently, some species of *Citrus* native to Indonesia and introduced species are commonly cultivated in Indonesia, namely *C. x aurantiifolia, C. grandis, C. hystrix, Citrus x aurantium* L., *Citrus medica* L., *Citrus* × *aurantium* f. *deliciosa*, *Citrus maxima* (Burm.) Merr. and *Citrus* crosses, such as *tangelo, tangor, citrumello,* and *citrange* (Horowidi et al. 2021). Some species of *Citrus* are also cultivated throughout Sulawesi specifically in South and West Sulawesi (Zulkarnain 2017).

Tomini Bay is the largest bay in Indonesia, bordered by Central Sulawesi, North Sulawesi, and Gorontalo provinces. The area is approximately 6,000,000 ha (Pramudji et al. 2019). Based on a preliminary survey in Tomini Bay in early February 2022, *Citrus* plants grow wild in yards, gardens or forests near resident's settlements, without being cultivated. Utilization of *Citrus* plants that are not balanced with the cultivation business will be detrimental to the availability of *Citrus.* Until now, the conservation and sustainable use of biodiversity, such as fruits, has not been optimal and is predicted to get worse (Harris et al. 2022). Thus, the first step to ensure the sustainability and diversity of *Citrus* in Tomini Bay is the investigation of genetic diversity using a biological approach by identifying morphological characters.

Identification of morphological characters has become the primary evidence for broad taxonomy, which is frequently employed by beginners (Kusumawardani et al. 2019). Morphological characters are simpler to notice and employ than anatomical and molecular characters. Although molecular data can offer information on morphology, the roles of genes and proteins in determining morphology can only be understood if the plant's morphology and its parts are first known. Morphological character is thought to be an essential line to define the genus as the most significant indicator to visually identify plants (Widodo et al. 2014; Kusumawardani et al. 2019; Susetyarini et al. 2020).

Previous research related to the morphological characterization of *Citrus* has been carried out in Indonesia, such as Rezkianti and Lakani (2016) and Adelina et al. (2017) in the Poso District area, Central Sulawesi; Murtando et al. (2016) in Parigi Moutong District, Central Sulawesi; and Adlini and Umaroh (2021) in Batu Bara District, North Sumatra. However, research on the genetic diversity study based on morphological characters of the genus *Citrus* has never been conducted in the coastal area of Tomini Bay. Therefore, this research aimed to provide information on the genus *Citrus* diversity in the coastal area of Tomini Bay for maintaining the sustainability of genetic resources to support the breeding program and conservation.

MATERIALS AND METHODS

Research sites

This research was conducted from June to August 2022. Sample exploration and collection of the genus *Citrus* was carried out around the coastal area of Tomini Bay which is located in Gorontalo Province that covers Gorontalo City, Gorontalo, Boalemo, Bone Bolango, and Pohuwato Regencies. This research was also carried out in Tojo Una-una Regency, Central Sulawesi (Figure 1 and Table 1). Observations of morphological characters, trait weighting, and PCA (Principal Component Analysis) analysis were carried out at the Botany Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Gorontalo, Indonesia.

Plant materials

The materials used in this study were 39 accessions consisting of 7 species of *Citrus* that were found spread in many villages in the coastal area of Tomini Bay (Table 1). The identified accessions are mostly plants growing in people’s house yards and open lands and few accessions are found cultivated in plantation areas.

Procedures

Sampling and data collection

Samples were collected from the field using a purposive sampling method with consideration of the availability of *Citrus* plants at that location. Each place explored was taken ± 3 plant accessions based on differences in morphological characters. Description in the field is done by recording initial information of *Citrus* plants or known plant passports (field notes) in the form of the collector's name, number and date of collection, local name, species name, stature, habitus, and sample origin status. In addition, vegetative organs (stems and leaves) and generative organs (flowers, fruits, and seeds) were observed, photographed, and measured (Febriyanti 2016; Sariamanah et al. 2016). Then, samples were labeled based on the abbreviated name of the district where the sample originated.

Identification of morphological characters

Samples obtained from the collection in the field are then identified by matching morphological data with descriptions in the literature or based on images. Morphological data collection based on *Citrus* plant descriptors listed in Descriptors for *Citrus* published by the International Plant Genetic Resources (IPGRI 1999) (Table 2).

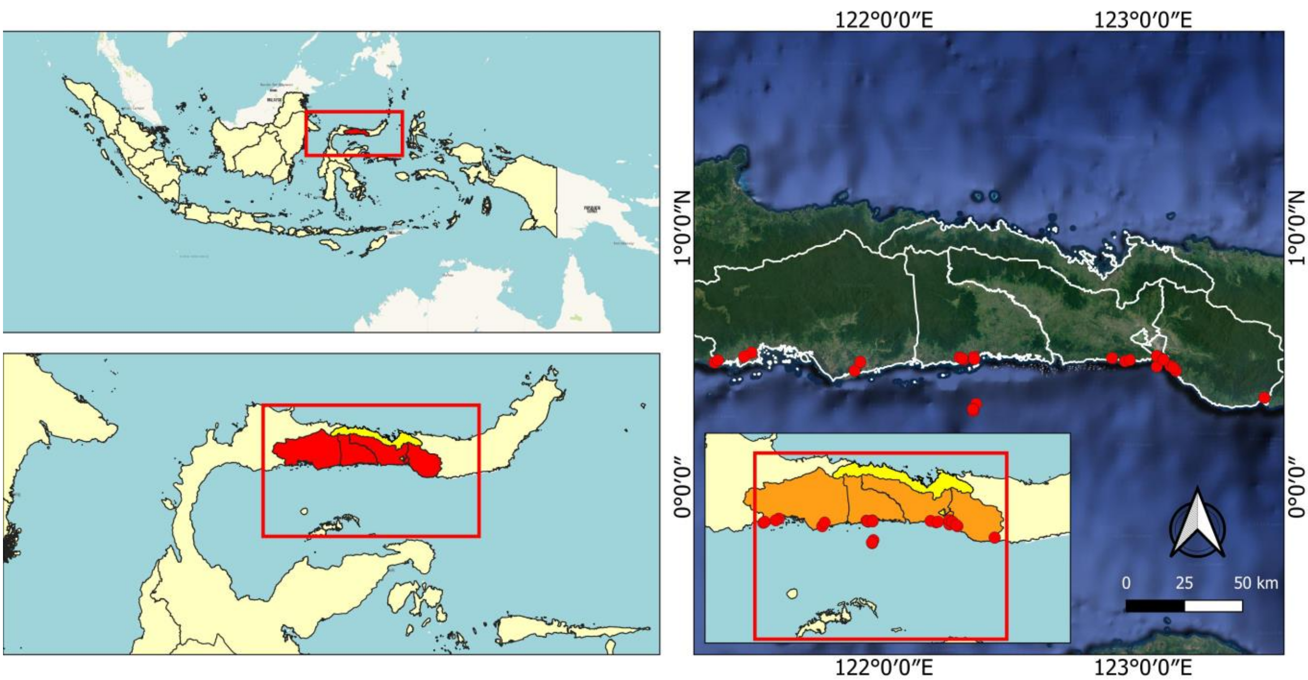

Figure 1. Research site in the coastal area of Tomini Bay, Gorontalo Province, Indonesia

Table 1. Distribution of *Citrus* plants in the coastal area of Tomini Bay, Gorontalo Province, Indonesia

Species	Local name	District/ city	Subdistrict	Village	Plant age (years)	Accession code	Altitude (m asl.)	Coordinates (Lat: N, Long: E)
Citrus × *limon* (L.) Osbeck	*Limu tutu/ lemon susu*	Gorontalo City	Hulonthalangi	Pohe	10	CKTGRTL 001	70.2	0030’23.7”; 123003’14.9”
		Gorontalo City	Dumbo Raya	Leato Selatan	4	CKTGRTL 006	72.6	0029’29.8”; 123004’53.2”
		Gorontalo City	Dumbo Raya	Leato Selatan	7	CKTGRTL 007	72.5	0029’33.0”; 123004’48.2”
		Bone Bolango	Kabila	Biluango	10	CKBBNBL 002	70.6	0027’43.4”; 123007’01.3”
		Boalemo	Tilamuta	Bajo	12	CKBBLMO 002	66.0	0029’41.9”; 122021’00.8”
		Pohuwato	Popayato	Molosipat	9	CKKPHWT 001	17.5	0028’54.7”; 121020’50.3”
		Pohuwato	Popayato Barat	Persatuan	12	CKKPHWT 004	19.9	0029’24.3”; 121021’34.2”
		Gorontalo	Batudaa Pantai	Biluhu Timur	11	CKBLHU 001	75.4	0029’30.0”; 122057’00.3”
		Tojo Una-Una	Walea Besar	Pasokan	8	CKTJUUN 002	61.8	0018’05.2”; 122020’39.6”
Citrus x aurantium L.	*Limu Cina*	Gorontalo City	Hulonthalangi	Pohe	7	CKTGRTL 002	70	0029’29.8”; 123004’53.0”
		Bone Bolango	Kabila	Botutonuo	3	CKBBNBL 004	8.9	0027’00.4”; 123007’39.0”
		Gorontalo	Batudaa Pantai	Tontayuo	12	CKBLHU 002	86.7	0029’13.2”; 122055’53.2”
Citrus hystrix DC.	*Limu pulu/ lemon purung*	Gorontalo City	Hulonthalangi	Pohe	3	CKTGRTL 003	70.2	0030’23.0”; 123003’14.6”
		Gorontalo City	Dumbo Raya	Leato Utara	3,5	CKTGRTL 005	72.3	0029’52.3”; 123004’16.2”
		Gorontalo City	Dumbo Raya	Leato Selatan	2	CKTGRTL 009	153.9	0029’37.8”; 123004’43.5”
		Bone Bolango	Kabila	Botutonuo	10	CKBBNBL 003	67.4	0027’00.2”; 121053’15.6”
		Pohuwato	Duhiadaa	Mootilango	12	CKKPHWT 007	11.1	0027’58.3”; 123003’14.9”
		Gorontalo	Batudaa Pantai	Tontayuo	13	CKBLHU 003	94.4	0029’13.2”; 122055’53.4”
		Tojo Una-Una	Walea Besar	Kondongan	15	CKTJUUN 005	116.2	0019’20.4”; 122021’24.1”
Citrus × *aurantiifolia* (Christm.) Swingle	*Limu kapati*	Gorontalo City	Hulonthalangi	Pohe	3	CKTGRTL 004	70.09	0030’23.3”; 123003’14.9”
		Gorontalo City	Dumbo Raya	Leato Selatan	4	CKTGRTL 008	72.2	0029’33.9”; 123004’46.9”
		Bone Bolango	Kabila	Huangobotu	3	CKBBNBL 001	69.2	0028’05.9”; 123006’22.8”
		Boalemo	Tilamuta	Pentadu Barat	4	CKBBLMO 001	67.9	0030’13.2”; 122020’52.1”
		Boalemo	Botumoito	Botumoito	10	CKBBLMO 004	66.2	0029’32.3”; 122018’31.4”
		Pohuwato	Popayato Barat	Persatuan	7,5	CKKPHWT 003	19.9	0029’24.3”; 121021’34.2”
		Tojo Una-Una	Walea Besar	Pasokan	20	CKTJUUN 004	73.6	0017’41.5”; 122020’44.2”
Citrus x microcarpa Bunge	*Limu cui*	Bone Bolango	Bone	Ilohuuwa	10	CKBBNBL 005	80.37	0020’44.9”; 123028’09.1”
		Boalemo	Botumoito	Potanga	3	CKBBLMO 005	8.2	0030’03.3”; 122018’46.6”
		Boalemo	Botumoito	Tutulo	5	CKBBLMO 006	6,4	0029’43.8”; 122018’15.9”
		Pohuwato	Popayato Barat	Molosipat	5	CKKPHWT 002	20	0029’24.4”; 121021’34.3”
		Pohuwato	Duhiadaa	Buntulia Barat	5	CKKPHWT 008	23.6	0029’01.2”; 121054’35.7”
		Tojo Una-Una	Walea Besar	Pasokan	4,5	CKTJUUN 001	72.6	0018’04.0”; 122020’40.9”
Citrus maxima (Burm.) Merr.	*Limu bongo/ limu kalapa/ lemon Jawa*	Bone Bolango	Bone	Taludaa	10	CKBBNBL 006	80.37	0020’44.9”; 123028’09.1”
		Boalemo	Botumoito	Tutulo	7	CKBBLMO 003	92.2	0030’03.0”; 122017’35.9”
		Boalemo	Botumoito	Botumoito	20	CKBBLMO 007	9.2	0029’50.3”” 122018’11.5”
		Pohuwato	Popayato	Maleo	5	CKKPHWT 006	55.1	0031’10.2”; 121029’23.2”
		Gorontalo	Batudaa Pantai	Olimo’o	20	CKBLHU 004	63.6	0029’57.0”; 122052’52.5”
		Tojo Una-Una	Walea Besar	Pasokan	10	CKTJUUN 003	67.3	0018’08.9”; 122020’42.7”
Citrus medica L.	*Limu olobu*	Pohuwato	Popayato	Telaga Biru	6	CKKPHWT 005	90.1	0030’15.8”; 121027’38.9”

Data analysis

Data on morphological characteristics of the genus *Citrus* were analyzed descriptively and delivered in the form of pictures and data tabulation. Diversity index, including richness and evenness was calculated using Shannon-Wiener index (Omayio and Mzungu 2019; Goswami et al. 2022). According to Peng et al. (2018), the formula of Shannon-Wiener's index is as follows:

$$H = -\sum_{i=1}^{s} \frac{n_i}{N} \ln \frac{n_i}{N}$$

Where:

H : the diversity in a circle of *S* species,

n_i : the number of individuals of the *i*th species,

N : the total number of individuals of all the species, and *ln* is the natural logarithm.

Higher H values indicate more species richness as well as the roughly equal abundance of all species within a quadrat or community. The categorization of Shannon-Wiener diversity index is presented in Table 3 (Peng et al. 2018).

Determining the closeness of kinship between two groups of plants can be seen from phenetic kinship. Phenetic kinship is based on the similarity of traits possessed by each group of plants without regard to their ancestral history. Kinship is very important because it can help to classify several taxonomic species (Stuessy 1990). Appropriate scoring of the observed quantitative and qualitative characters was standardized using the Multivariate Statistical Program (MVSP) software version 3.1 pc. Data analysis was carried out on MVSP (Multivariate Statistical Package) v.3.1 software to construct phenetic dendrograms and Principal Component Analysis (PCA). The Gower's General Similarity Coefficient and Unweighted Pair Group with Mean of Arithmetic (UPGMA) clustering methods were used to create the dendrogram, while the Euclidean Distance algorithm was used to create the PCA scattered plot. Furthermore, PCA was also used to define the role of each morphological character in accession grouping (Sari et al. 2016; Windiyani et al. 2022).

Table 2. List of qualitative and quantitative characters for *Citrus* (*Citrus* spp.) according to IPGRI (1999)

Characters	Methods of assessment and scaled used
Qualitative characters	
Vegetative characters	
Stem	
Ration trunk diameter	Smaller (<1) (1), same (1) (2), larger (> 1) (3)
Trunk surface	Smooth (1), grooved and ridged (2)
Tree shape	Ellipsoid (1), spheroid (2), obloid (3), other (99)
Tree growth habit	Erect (1), spreading (2), drooping (3), other (99)
Density of branches	Sparse (3), medium (5), dense (7)
Branch angle	Narrow (3), medium (5), wide (7)
Spine density on adult tree	Absent (0), low (3), medium (5), high (7)
Spine length on adult tree	≤ 5 mm (1), 6-15 mm (2), 16-40 mm (3), >40 mm (4)
Spine shape	Curved (1), straight (2)
Shoot tip color	Green (1), purple (2), other (99)
Shoot tip surface	Glabrous (1), intermediate (2), pubescent (3)
Leaf	
Vegetative life cycle	Evergreen (1), deciduous (2), semi-persistent (3)
Leaf division	Simple (1), bifoliate (2), trifoliate (3), pentafoliate (4), other (99)
Intensity of green color of leaf blade	Light (1), medium (green) (2), dark (3)
Leaf color variegation	Absent (0), present (1)
Leaf lamina attachment	Sessile (1), brevipetiolate (2), longipetiolate (3)
Leaf lamina shape	Elliptic (1), ovate (2), obovate (3), lanceolate (4), orbicular (5), obcordate (6), other (99)
Leaf lamina margin	Crenate (1), dentate (2), entire (3), sinuate (4), other (99)
Leaf apex	Attenuate (1), acuminate (2), acute (3), obtuse (4), rounded (5), emarginate (6), other (99)
Absence/presence of petiole wing	Absent (0), present (1)
Petiole wing width	Narrow (3), medium (5), broad (7)
Petiole wing shape	Obcordate (1), obdeltate (2), obovate (3), linear (4), other (99)
Junction between petiole and lamina	Fused (1), articulate (2)
Color of leaf upper/lower surface	Same (1), lighter (2), darker (3)
Nerves on leaf upper surface	Protuberant (1), flat (2)
Angle of leaf bases	Acute (1), obtuse (2)
Angle of leaf apex	Acute (1), obtuse (2)
Petiole attachment to twigs	Straight (1), curved (2)

Generative characters	
Flower	
Calyx diameter	Small (3), medium (5), large (7)
Length of anthers relative to stigma	Shorter (3), medium (5)
Flower type	Hermaphrodite (1), male (2), female (3), other (99)
Color of open flower	White (1), light yellow (2), yellow (3), purple (4), other (99)
Color of anthers	White (1), pale yellow (2), yellow (3), other (99)
Number of stamens	< 4 per petal (1), 4 per petal (2), > 4 per petal (3)
Arrangement of flowers	Solitary (1), inflorescence (2), both (3)
Flower/inflorescence position	Axillary (1), terminal (2), both (3)
Stamina length	Short (3), medium (5), long (7)
Separation of filament	Separated (1), partially united (2), united (3)
Style shape	Straight (1), arched (2), crooked (3)
Bearing habit	Inside canopy (1), outside (2), both (3)
Fruit	
Fruit shape	Spheroid (1), ellipsoid (2), pyriform (3), oblique (asymmetric) (4), obloid (5), ovoid (6), other (99)
Shape of fruit base	Necked (1), convex (2), truncate (3), concave (4), concave collared (5), collared with neck (6), other (99)
Shape of fruit apex	Mammiform (1), acute (2), rounded (3), truncate (4), depressed (5), other (99)
Fruit skin (epicarp) color	Green (1), green-yellow (2), light-yellow (3), yellow (4), dark yellow (5), light orange (6), orange (7), dark orange (8), pink-yellow (9), pink-orange (10), red (11), red-orange (12), other (99)
Fruit surface texture	Smooth (1), rough (2), papillate (3), pitted (4), bumpy (5), grooved (6), other (99)
Adherence of albedo (mesocarp) to pulp (endocarp)	Weak (3), medium (5), strong (7)
Nature (conspicuousness) of oil glands	Inconspicuous (1), conspicuous (2), strongly conspicuous (3)
Density of oil gland on fruit surface	Low (<40/cm^2) (3), intermediate (45-65/cm^2) (5), high (>70/cm^2) (7)
Oil gland size on fruit surface	Small (<0.8 mm) (3), large (≥1.2 mm) (7)
Albedo color	Greenish (1), white (2), yellow (3), pink (4), orange (5), reddish (6), other (99)
Absence/presence of areola	Absent (0), present (1)
Fruit stylar end	Closed (1), open (2), persistent style (3), other (99)
Fruit attachment to stalk	Weak (3), medium (5), strong (7)
Number of segments per fruit	< 5 (1); 5-9 (2); 10-14 (3);15-18 (4); >18 (5)
Adherence of segment walls to each other	Weak (3), medium (5), strong (7)
Segment shape uniformity	No (0), yes (1)
Thickness of segment walls	Thin (3), medium (5), thick (7)
Fruit axis	Solid (1), semi-hollow (2), hollow (3)
Cross-section shape of axis	Round (1), irregular (2)
Pulp	
Pulp (flesh) color	White (1), green (2), yellow (3), orange (4), pink (5), light-red (6), orange-red (7), red (8), purple (9), other (99)
Pulp color intensity	Light (3), dark (7)
Pulp color uniformity	No (streaked) (0), yes (1)
Pulp firmness	Soft (3), intermediate (5), firm (7)
Pulp texture	Crispy (1), fibrous (2), fleshy (3), other (99)
Vesicle length	Short (3), medium (5), long (7)
Vesicle thickness	Thin (3), medium (5), thick (7)
Juice content in endocarp	Low (3), medium (5), high (7)
Seed	
Average number of seeds per fruit	None (0), 1-4 (1), 5-9 (2), 10-19 (3), 20-50 (4), >50 (5)
Seed shape	Fusiform (1), clavate (2), cuneiform (3), ovoid (4), semi-deltoid (5), spheroid (6), semi-spheroid (7), other (99)
Seed surface	Smooth (1), wrinkled (2), hairy (3), other (99)
Seed color	White (1), cream (2), yellowish (3), green (4), brown (5), other (99)
Cotyledon color	White (1), light yellow-cream (2), light green (3), white and green (4), green (medium) (5), dark green (6), purple (7), pinkish (8), other (99)
Chalazal spot color	White (1), cream (2), yellow (3), light brown (beige) (4), brown (5), reddish (6), purple (7), other (99)
Quantitative characters	
Generative characters	
Plant height (m)	From ground level up to the highest point of canopy
Canopy diameter (m)	At the widest point
Leaf lamina length (cm)	From petiole base to lamina tip, average of 10 fully developed leaves taken from three different adult trees
Leaf lamina width (cm)	At the widest point, average of 10 fully developed leaves taken from three different adult trees
Leaf lamina length/width ratio	Average of 10 fully developed leaves taken from three different adult trees
Leaf thickness (mm)	At the thickest point, average of 10 fully developed leaves taken from three different adult trees

Vegetative characters	
Pedicel length (mm)	Average
Petiole length (mm)	0-10 (1), 11-15 (2), >15 (3)
Number of petals per flower	Count
Petal length (mm)	Average
Petal width (mm)	Average
Number of flower buds in inflorescence	Count
Length of anthers (mm)	Average
Fruit weight (g)	Average
Fruit diameter (mm)	Average
Fruit length (mm)	Average
Fruit skin thickness (mm)	Equatorial area
Diameter of fruit axis (mm)	Average
Seed length (mm)	Average
Seed width (mm)	Average
Seed weight (g)	Average

Table 3. Value and description of the Shannon-Wiener diversity index (H')

H' < 1.0	1.0 < H' < 3.22	H' > 3.22
Low diversity	Moderate diversity	High diversity
Low productivity	Medium productivity	High productivity
Unstable ecosystems	Balanced ecosystem	Stable ecosystem

RESULTS AND DISCUSSION

Morphological characters

Plant morphology plays a vital role as the first step to plant identification. The observed morphological aspects in a plant group are plant height, shapes or color of flowers, fruits, seeds, and so on. Morphological marker is used as the foremost tools before continually using other methods. The use of morphological characters in this study is perceived important as it can be used in all levels of the hierarchical taxonomy and it plays a bigger role in determining the species and other categories under the species (Febriyanti 2016; Ahmad et al. 2020). Environmental variables influence the differences in the morphological traits of plant populations in a region. Altitude, rainfall, temperature, and humidity are examples of environmental variables that encourage plant growth and can have an impact on the morphological traits of populations of related plants (Ismail et al. 2019; Tolangara et al. 2020). The morphological characters of *Citrus* in the coastal area of Tomini Bay are presented in Figure 2.

Growth and stem characters

The results showed diversity in growth and stem characters. The height and canopy diameter at maturity of the genus *Citrus* ranges from 2.2 to 11.55 m and 2.02 to 7.6 m, respectively with a diameter ratio of more than 1. The most dominant growth habit of a tree is spreading while the least discovered is drooping on several accessions, such as CKTGRTL 003, CKTGRTL 006, CKBBLMO 005, and CKKPWHT 007. The most dominant tree shape is ellipsoid, while the least discovered tree shape is spheroid on accessions CKTGRTL 002, CKTGRTL 007, CKBLHU 001, and CKBLHU 004. The stem surface is dominated by notched and wrinkled surfaces. CKTGRTL 004, CKTGRTL 008, CKKPHWT 007, and CKBLHU 004 are ones with smooth stem surfaces. The branch density was considered sparse on some accessions, such as CKTGRTL 003, CKTGRTL 004, CKBBLMO 003, CKBBLMO 006, CKKPHWT 002, CKKPHWT 006, CKTJUUN 001, and CKTJUUN 002. The most dominant branch angle was medium while the least discovered angle was wide on several successions, such as CKTGRTL 009, CKKPHWT 006, CKTJUUN 001, and CKTJUUN 002. There are at least 4 variations on the parameter of spine density on adult trees, such as absent, low, medium, and high. The most dominant spine density is categorized as low. The spine length of an adult tree ranges from 2.8 to 50 mm with uneven spread. The spine shapes are found to vary from straight to curved. Almost all studied cultivars possessed green shoot tip color except CKTGRTL 003 with brown color. The most dominant shoot tip surface is glabrous. Accessions CKBLHU 004 and CKTJUUN 003 are only *C. maxima* with fury shoot tip. Yet there were also found some species of *C. maxima* with glabrous shoot tips.

Morphological diversity ranges from size, shape, angle, and type of branching, as well as the position of leaves and flowers that form plant architecture. Plant architecture is the most reliable standard for taxonomic classification and has a major impact on determining the suitability of a plant for efficient cultivation, production, and harvesting (Reinhardt and Kuhlemeier 2002; Setyawan et al. 2021). In addition, seed number, seed polyembryony, tree growth habit, and tolerance to biotic and abiotic stresses, tree shape also play an important role in plant breeding (Yulianti et al. 2020). Plant architecture, such as branching is affected by both genetic and environmental influences. Genetic aspects are regulated by a large number of genes (Cai et al. 2016; Mauro-Herrera and Doust 2016). In addition, as the tree ages, the weight of the tree itself as well as external factors such as wind, snow, and light have a significant impact on its shape. Trees can dynamically adjust their shape to cope with mechanical stress as the surrounding environment changes over time (Tsugawa et al. 2022).

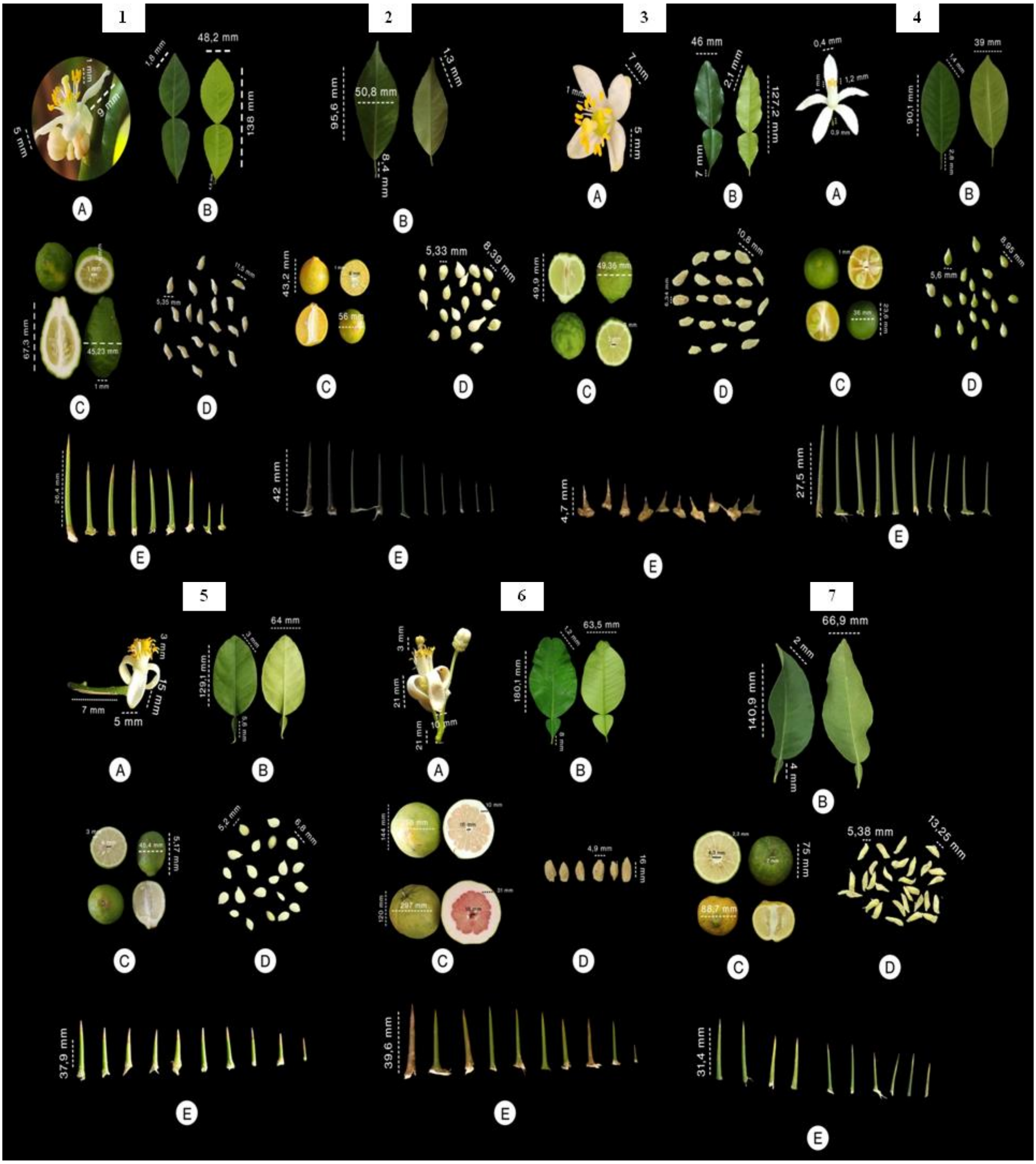

Figure 2. Morphological characters of *Citrus* in the coastal area of Tomini Bay: (1) *C.x limon*, (2) *C. x aurantium*, (3) *C. hystrix*, (4) *C. x aurantiifolia*, (5) *C. x microcarpa*, (6) *C. maxima*, and (7) *C. medica*. A = flower, B = leaf, C = fruit, D = seed, E = spine

Spines are modified leaves with a sharp tip structure and are located on the branches or trunks of *Citrus* plants. These structures can protect the plant from herbivore bites and prevent water loss within the tissue (Harris and Harris 2006; Mares 2017). In this study, not all *Citrus* plants had spines, even within the same species, such as in *C. microcarpa* and *C. maxima*. This is in line with Budiarto et al. (2021) which found that *C. microcarpa* does not have spines. The absence of spines on *Citrus* plants may be influenced by the age of the tree. According to Wu et al. (2021a), most *Citrus* species have spiny branches during the juvenile stage and new branches growing from mature trees usually have no spines or are much fewer and shorter. The presence of spines on *Citrus* plants is sometimes detrimental to farmers, because it can increase production costs during the process of handing over products after harvesting, moving, and maintaining in the field (Santos et al. 2015). Habitus of *Citrus* in the coastal area of Tomini Bay is presented in Figure 3.

Leaf characters

The results showed diversity in leaf characters. The intensity of the green color of leaves found in this research possessed 3 variations, namely light to medium and dark. Color variations are also found on the leaf surface. The attachment shape of the leaflets of all genus *Citrus* found in this research is brevipetiolate. The longest leaflet was found on accession CKBBLMO 007 of 180.1 mm, while the shortest leaflet was found on accession CKTJUUN 001 of 56.3 mm. The largest accession was found on accession CKTGRTL 006 of 80 mm. The narrowest was found on the accession CKTJUUN 001 of 25.5 mm. Thus, it can be concluded that *C. microcarpa* has the smallest leaves. On the parameter of length and width ratio of the leaflet, the highest ratio was found on accession CKKPHWT 001 as big as 4.72, while the lowest was found on accession CKTJUUN 004 as big as 1.77. The leaf thickness of the genus *Citrus* is around 1 to 3.94 mm. On the leaf shapes parameter, 5 variations were found, namely elliptic, ovate, obovate, orbicular, and obcordate. The most dominant shape is ovate. On the parameter of leaf lamina margin, 5 variations were found, such as acuminate, acute, obtuse, rounded, and emarginate. Almost all genus *Citrus* have petiole wings except on 15 other accessions. The width of the petiole wings is varied from narrow, medium, to broad. The shape of petiole wings is also varied from obcordate, obdeltate, and obovate. The least shape of the petiole wings is obdeltate. The most dominant junction between petiole and lamina is fused while the least discovered is articulated on accessions CKTGRTL 002, CKBBNBL 005, CKKPHWT 002, dan CKTJUUN 003. The colors of leaf surface in which the upper surface is darker. Nerves on the upper leaf surface are divided into two kinds, i,e, protuberant and flat. Petiole attachment to twigs are divided into two: curved and straight. The leaf base angle and apex are also divided into two namely obtuse and acute. The length of the stalk is around 0.42 to 15.5 mm.

Citrus species never shed their leaves during the vegetative period, unless they are old or stressed, tend to have thicker leaves. Green leaves are correlated with many structural and functional characteristics of leaves (Yunus et al. 2018; Prasad et al. 2021). The simple shape of *Citrus* leaves is in line with Sofiyanti et al. (2022), that in general, the leaves are subcoriaceous, alternately arranged, simple leaf or 1-folio-late laminae (also called unifoliate). Brevipetiolate is a shape where the branch is shorter than the leaflet (IPGRI 1999; Budiarto et al. 2021; Chuenwarin et al. 2021).

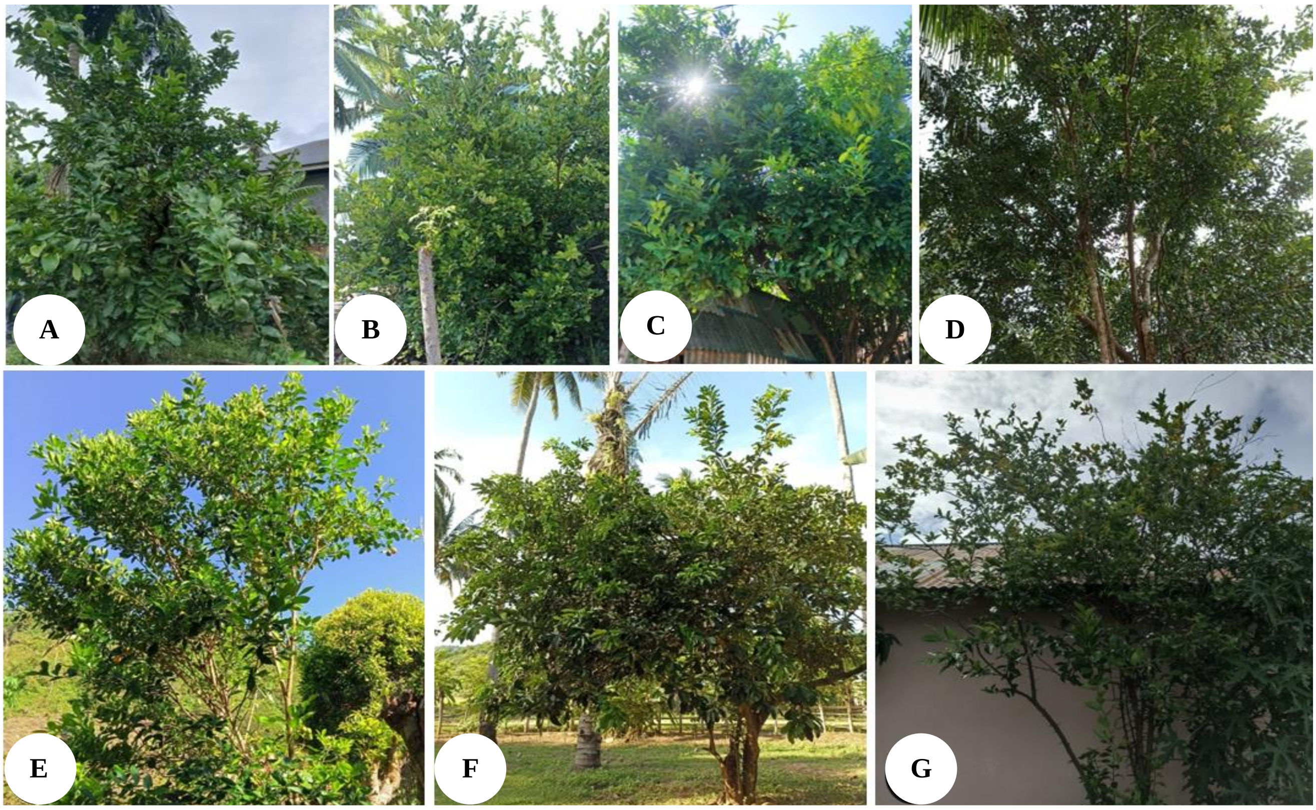

Figure 3. Habitus of *Citrus* in the coastal area of Tomini Bay: A. *C. medica*, B. *C. x aurantiifolia*, C. *C. x limon*, D. *C. hystrix*, E. *C. x macrocarpa*, F. *C. maxima*, and G. *C. x aurantium*

Leaf morphology can be determined by cytokinin activity, whereas leaf area is generally determined by the number and size of cells in the leaf. Cytokinins promote cell division and increase cell expansion during the proliferation and expansion stages of leaf cell development. During leaf senescence, cytokinins reduce sugar accumulation, increase chlorophyll synthesis, and prolong the photosynthetic period of leaves (Wu et al. 2021b). According to Wang et al. (2019), leaf size affects the cycles of carbon, water, and energy. The small size of the leaves can accelerate the loss of water in the leaves through the transpiration process. Besides, the area of a leaf also impacts photosynthesis. The wider the leaf, the easier it is to capture the sunlight (Yunus et al. 2018). The leaf length ratio to the leaf width is organized by several genetic factors. Mutation may probably support the evolution of leaf shapes. However, not all shapes can be explained by environmental adaptation (Tsukaya 2018). In addition to leaf size, the presence or absence of petiole wings is predictably caused by genetic factors. They only appear as a line that stands out on the surface of the existing leaf stalk (Budiarto et al. 2021).

We may conclude that *Citrus* trees have varied leaf shapes based on the findings. Genetic and environmental variables both have an impact on how differently shaped leaves differ from one another. According to Dkhar and Pareek (2014), a variety of genetic interactions, alterations in gene expression patterns, microRNA involvement, and active hormonal regulation, some of which are reprocessed during leaf development or type specification, regulate leaf development and the various forms it acquires. Environmental variables, in addition to genetic regulation, are crucial during the final modification of leaf form. Similarly, the environment in which a plant develops and elements like the availability of food, water, and light all have an impact on leaf color variation (Chan et al. 2022).

Flower characters

The longest flower stalk length was on accession CKBLHU 004 of 21 mm, while the shortest flower was found on accession CKKPHWT 003 and CKTJUUN 004. Calyx diameters are varied from small, medium, and big. All species within the genus *Citrus* have white hermaphrodite flowers and yellow pollens. The total petals (corolla) were 4-5. On the parameter of petal length, the longest one was found on accession CKBBNBL 006 of 23 mm, while the shortest one was found on accessions CKTGRTL 006 and CKBLHU 001 of 3 mm The largest petal width was on accession CKBBLMO 007 of 21 mm, while the narrowest petal width was CKBBNBL 002 of 3 mm. Of all the species, the total number of stamens found was more than 4. The length of the stamens are varied from short, medium, to tall. The filament divisions of stamens are divided into three parts, namely separated, partially united, and united. The length of the anther is 0.1 to 4 mm. The placement of the flower can be found in two positions, in the leaf axial and apical.

Among the *Citrus* plants, the blooming season and its intensity is highly complex. Due to its mortal nature, the plants interact with the environmental condition for a year long. The flowering stage occurs according to the species, age of trees, and climate conditions, such as rainfall, temperature, radiation, and dried threats. A tree can produce 250,000 flowers yet there is only less than 1% that grows mature fruits. *C. x limon* and other cultivars can bloom flowers once or more in a year (regular bearing), while the rest of them can abundantly bloom flowers in a year then it is followed by decreased numbers of flowers or not at all in the following blooming season of the year (alternate bearing) (Suaria et al. 2021; Agustí et al. 2022).

The floral characters revealed in this study are in line with studies conducted on the flowers of *C. x microcarpa* (Mapalo and Rosillo-Magno 2018), *C. maxima* (Hoque 2015; Sedeek et al. 2017), *C. x limon* (Szczykutowicz et al. 2020), *C. x sinensis* (Favela-Hernández et al. 2016), *C. hystrix* (Agouillal et al. 2017), *C. x aurantiifolia* (Kumari et al. 2021), and *C. medica* (Rani and Gill 2020). In general, the flowers are white, bisexual, have 4-5 petals, are arranged in the axils, usually solitary or occasionally appear in small corymbs with a diameter of about 1.5-2.5 cm, petals are white to white-pink with many stamens and usually have a strong aroma, the stigma is usually round, and appears light yellow to yellowish, filaments are light green. The stigma is in the center of each flower and is rounded around the stamens which consist of long filaments with yellow pollen grains on them (Gurung and Chetri 2021).

Fruit characters

Accession CKTJUUN 003 has the heaviest fruit weight of 1.95 kg, while accession CKBBLMO 006 has the lightest fruit weight of 7.6 g. Fruit length ranges between 2.36 and 150 mm. Fruit diameter ranges between 0.9 and 88.7 mm. On the parameter of fruit shapes, 4 shapes were found as ellipsoid, obloid, spheroid, and pyriform. The least fruit shape was found on accession CKTGRTL 003 and pyriform was found on accession CKKPHWT 006. On the parameter of the base shape of fruit, there were 5 variations found, such as necked, convex, truncate, concave, and concave collared. The most dominant shape was necked and the least popular shape was concavely collared which was found on accession CKTJUUN 003. On the parameter of fruit apex, 4 variations were found such as mammiform, rounded, truncate, and depressed. The colors of epicarp of all species are green and yellowish-green. On the parameter of fruit surface texture, 6 variations were found, i.e. smooth, rough, papillate, pitted, bumpy, and grooved. Papillate texture was only found on accession CKBBNBL 004. On the parameter of oil glands, there were striking and ordinary glands and varied in the density of oil glands starting from low ($<40/cm^2$), intermediate ($45-65/cm^2$), and high ($>70/cm^2$). Fruit thicknesses are also varied from low ($<40/cm^2$), intermediate ($45-65/cm^2$), and high ($>70/cm^2$). Fruit thickness is varied from 0.3 to 6 mm. Albedo's color is varied from white, greenish, yellow, and pink. *Citrus* navels are varied from closed, opened, and persistent style navels. Fruit attachments on fruit stalks are grouped into some forms such as weak, medium, and strong.

The location of bud that produces flowers and fruits are located inside and outside the canopy. The total segments per fruit are around 5 to 14 segments. The attachment of one and the other segment wall is divided into weak, medium, and strong. The segment color of *Citrus* is not always similar to the thickness as it varies from thin, medium, and thick. There is empty, hollow, and solid rachis. An empty rachis was found on accession CKBLHU 004. Fruit axis cross-section has several shapes such as round and irregular. The diameter of rachis reaches 2 to 37 mm. Pulp colors are varied from green, yellow, orange, pink, and mustard with light color intensity. The pulp textures are fibrous and fleshy. The length of *Citrus* kernel, the thickness of *Citrus* kernel and the juice content on the mesocarp are varied from low, medium, and high. On the seeds parameter, the average total number of seeds per fruit is 1-50. There are 7 variations of seed shapes such as fusiform, clavate, cuneiform, ovoid, semi-deltoid, spheroid, and semi-spheroid with smooth or wrinkled seed surfaces. The cotyledon color found varied from beige, white, green, brown, and yellow. Seed length ranges between 5.16 and 16.4 mm, seed width ranges from 1.29 to 6.2 mm, and seed weight ranges between 0.02 and 3.5 mm.

Citrus maxima is the biggest fruit of all species of *Citrus* with a thick and spongy mesocarp (Fayaz et al. 2020; Mohammed et al. 2021; Sofiyanti et al. 2022; Visakh et al. 2022). *C. microcarpa* and *Fortunella crassifolia* Swingle are classified as small *Citrus* fruits in Taiwan (Chen et al. 2017). Based on the results presented above, we can conclude that *Citrus* fruits have different morphologies. Fruit size and form are intricate characteristics that depend on a variety of variables, including genetic and environmental ones (temperature and humidity). Fruit morphological variations, including differences in size, shape, and color, are the consequence of adaptive evolution. Gene expression and/or gene function alterations are the fundamental processes underpinning modifications in plant morphology. Under natural circumstances, variation in fruit size and form has an evolutionary impact and frequently contributes to the isolation of species' reproductive systems. Furthermore, fruit form frequently changes after changes in size, suggesting that the two characteristics may have a shared set of genetic regulators. Fruit size is an important breeding goal (Wang et al. 2015; Li et al. 2022; Zhang et al. 2022).

Diversity index

Species diversity can be defined as diversity between species within an ecosystem (Kumar et al. 2022). Genus diversity can be seen from the number of plant species of the genus *Citrus* that existed in the research location calculated using Shannon-Wiener's index. The calculation result of the diversity index of the genus *Citrus* in the coastal area of Tomini Bay was H'= 0.8103 which is categorized as low with 7 species in total and 748 total individuals. The low diversity index is due to the domination of one species that is *C. x limon*. The diversity index of the genus *Citrus* is presented in Table 4.

Dominant species is a key to understanding the impacts of global change, predictions of community assembly, and studying ecological processes across hierarchical and spatial scales (Avolio et al. 2019). Dominant plants ameliorate the effect of the environment on nondominant instead while dominant plants deplete the available resources (Arnillas et al. 2021). According to Indriyanto (2012), a community is perceived to have a low diversity index if the community is made of a few species. The value of Shannon-Wiener diversity index is also correlated with the domination index value. The higher the domination index, the lower the diversity index (Aigbe and Omokhua 2015). The low level of diversity of plant species is caused by the vulnerability of the area where these plants live to various disturbances (Azizah 2017). The low level of plant species diversity indicates that *Citrus* plants in the coastal area of Tomini Bay are vulnerable to disturbances. According to Wahyuni et al. (2017), the variety of human activities in coastal areas makes this area the area most easily affected by human activities. Disruption to the stability of *Citrus* plants can be caused by human factors such as logging and land conversion. Furthermore, there is no *Citrus* cultivation by the community.

The community often utilizes the plant. In addition, the low diversity index is also influenced by habitat and environmental conditions. The temperature and altitude of the area where *Citrus* grow range from 24.81°C to 30.27°C and 6.4 to 116.2 m asl. which are classified as lowlands. Some species of *Citrus* can grow and adapt in the lowlands. However, *C. medica* is by far the most temperature sensitive of all *Citrus* species and so it is rarely found (Klein 2014). Therefore, attention is needed from the community and local government so that they are wise in managing the existing vegetation in the coastal area of Tomini Bay to conserve genetic resources.

Table 4. Diversity index of 7 species of *Citrus* in the coastal area of Tomini Bay

Species	Number of individuals per species	N	ni / N	pi ln pi	Category
Citrus x limon (L.) Osbeck	> 50	182	748	0.16875	-0.14935
Citrus x aurantium L.	> 50	104	748	0.09653	-0.11914
Citrus x aurantiifolia (Christm.) *Swingle*	> 50	119	748	0.11042	-0.12701
Citrus medica L.	< 50	36	748	0.04800	-0.06341
Citrus hystrix DC	> 50	85	748	0.07917	-0.10733
Citrus x microcarpa Bunge	> 50	92	748	0.08542	-0.11194
Citrus maxima (Burm.) Merr.	> 50	130	748	0.12083	-0.13208
Total Number of individuals		748	748	0.16875	-0.14935
					-0.8103
					H'= 0.8103

Phenetic relationships among *Citrus* spp. based on morphological characters

Kinship analysis was conducted on 39 accessions of *Citrus* which was found around the coastal area of Tomini Bay. The analysis result of kinship was presented in the form of a dendrogram of kinship (Figure 4). The analysis was carried out based on 63 representatives of morphological characters which had been predicted to be able to separate the observed species. Cluster analysis which was conducted by using the UPGMA method created two main clusters. Cluster analysis was used to analyze the diversity and classify plants based on data and identified morphological parameters.

Kinship is a relationship pattern or total similarities between groups based on certain characters of each group. The kinship approach used to determine the closeness of the relationship between two groups of the plant is the phenetic kinship (Suryadi et al. 2020). The observed characteristics are qualitative and quantitative. Qualitative characters are controlled by several genes which are easy to observe, while the differentiation of quantitative characteristics between genotypes is limited due to the influence of environmental factors (Perić et al. 2014). The reconstruction result of the dendrogram showed 2 main clusters. Both clusters group on a similarity coefficient of 64.3% (0.643). Cluster I consists of 9 *Citrus* accessions which are divided into two subclusters, namely subcluster IA consists of 1 accession, namely CKBBLHU-CMX, and subcluster IB consists of 8 accessions, i.e. CKBBLMO-CMX, CKTJUUN-CMX, CKKPHWT-CMX, CKBBLMO-CMX, CKBBNBL-CMX, CKTJUUN-CH, CKBLHU-CH, and CKTRGRTL-CH. These two subclusters is separated at a similarity coefficient of 66.3% (0.63) and had the same morphological characters in the form of scion trunk surface, tree shape, shoot tip surface, leaf lamina shape, shape of fruit base, oil gland size on the fruit surface, absence/presence of areola, the cross-section shape of axis. The morphological characters that differentiate between subclusters IA and IB are summarized in Table 5.

Table 5. Distinguishing characters between members of subclusters IA and IB in the grouping of accessions of the genus *Citrus*

Character	Subcluster IA	Subcluster IB
Scion trunk surface	Smooth	Grooved and ridged
Tree shape	Spheroid	Ellipsoid, obloid
Shoot tip surface	Pubescent	Glabrous, intermediate
Leaf lamina shape	Obcordate	Ovate, obovate, orbicular
Shape of fruit base	Absent	≤ 5-40 mm
Oil gland size on fruit surface	Small	Large
Absence/presence of areola	Present	Absent
Cross-section shape of axis	Round	Irregular

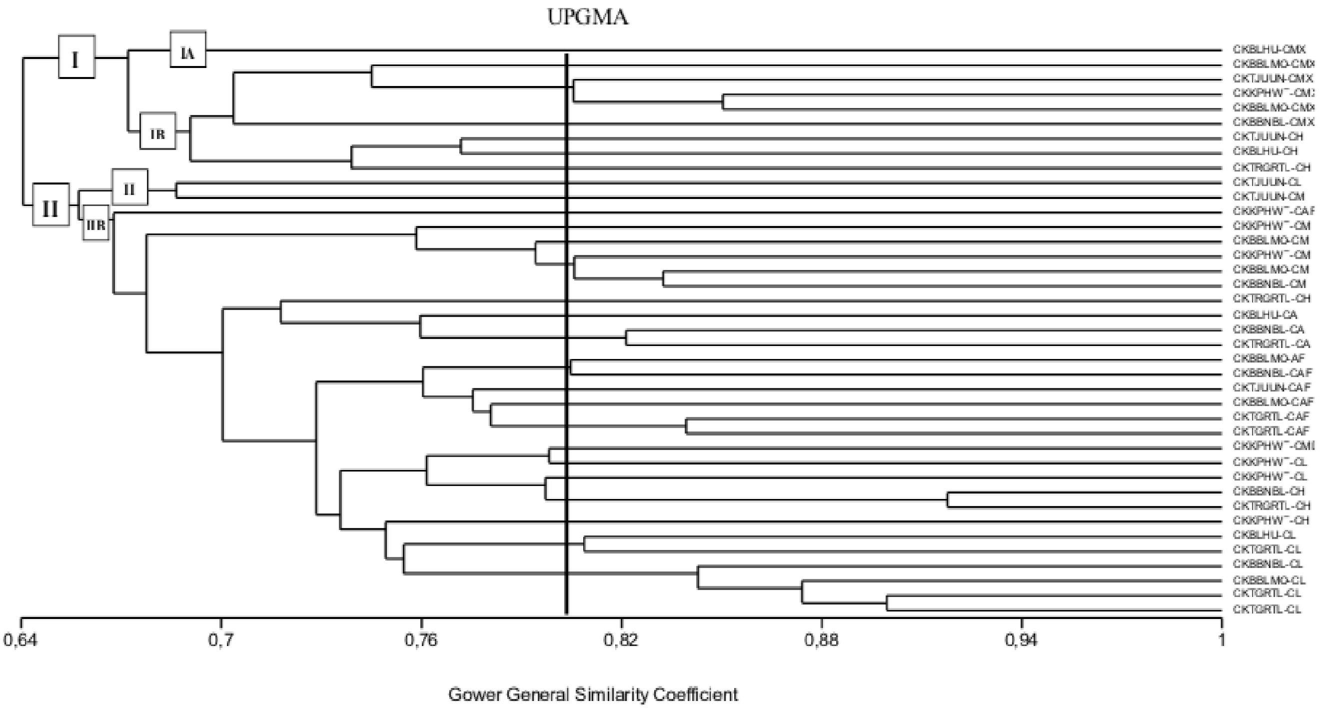

Figure 4. The dendrogram on the kinship of 39 accessions in 7 species of *Citrus* based on its morphological characters using the UPGMA method on the MVSP program. CH (*Citrus hystrix*), CA (*Citrus* × *aurantium*), CL (*Citrus* × *limon*), CAF (*Citrus* × *aurantiifolia*), CM (*Citrus* × *microcarpa*), CMX (*Citrus maxima*), CMD (*Citrus medica*)

Cluster II consists of 30 *Citrus* accessions which are grouped into 2 subclusters, namely subclusters IIA and IIB with a similarity coefficient of 65.7% (0.657). Sub-cluster II A consists of 2 accessions, namely CKTJUN-CL and CKTJUUN-CM, while subcluster IIB consists of 28 accessions and divided into subclusters IIBa and IIBb which separate at a similarity coefficient of 66.8% (0.668). Subcluster IIBa consists of only 1 accession, namely CKKPHWT-CAF and whereas subcluster IIBb consists of 27 accessions, namely CKKPHWT-CM, CKBBLMO-CM, CKKPHWT-CM, CKBBLMO-CM, CKBBNBL-CM, CKTRGRTLO-CH, CKBBLHU-CA, CKBBNBL-CA, CKTRGRTL-CA, CKBBLMO-CAF, CKBBNBL-CAF, CKTJUUN-CAF, CKBBLMO-CAF, CKTGRTL-CAF, CKTGRTL-CAF, CKKPHWT-CMD, CKKPHWT-CL, CKKPHWT-CL, CKBBNBL-CH, CKTRGRTL-CH, CKKPHWT-CH, CKBBLHU-CL, CKTGRTL-CL, CKBBNBL-CL, CKBBLMO-CL, CKTGRTL-CL, and CKTGRTL-CL. The 30 *Citrus* accessions in each of these subclusters have similarities in morphological form, namely density of branches, spine density, spine length and oil gland size on the fruit surface. The similarity of these distinguishing characters is presented in Table 6.

The cluster analysis showed a high level of similarity that shows intense closeness among 39 accessions found in the coastal area of Tomini Bay. It can be perceived that the original location does not determine the species grouping. Instead, morphological characteristics significantly determine the species grouping. This statement is in line with Manivannan et al. (2015) which showed that genotype grouping based on morphological characteristics does not always relate to the original location.

Principal component analysis

Principal component analysis in two-dimensional plots shows the same clustering results as the clustering pattern of *Citrus* accessions and the distribution pattern of morphological characters (Figure 5).

Table 6. Distinguishing characters between members of subcluster IIA and subcluster IIB in the grouping of accessions of the genus *Citrus*

Character	Subcluster IIA	Subcluster IIB
Density of branches	Dense	Sparse, medium
Spine density	Absent, high	Low, medium
Spine length	Absent	≤ 5-40 mm
Oil gland size on fruit surface	Large	Small

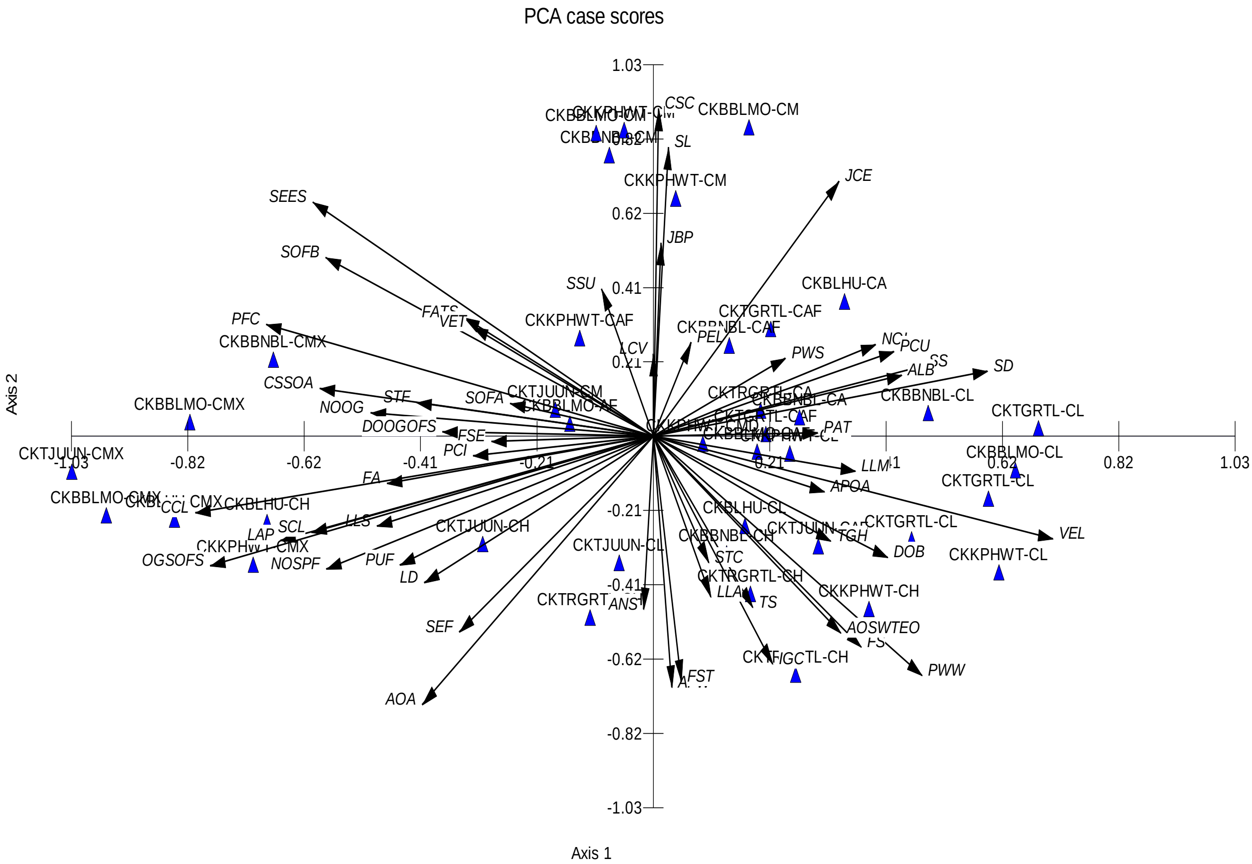

Figure 5. Grouping diagram of 39 *Citrus* accessions based on the distribution of morphological characters. Note: CH (*Citrus hystrix*), CA (*Citrus* × *aurantium*), CL (*Citrus* × *limon*), CAF (*Citrus* × *aurantiifolia*), CM (*Citrus* × *microcarpa*), CMX (*Citrus maxima*), CMD (*Citrus medica*)

Grouping patterns of the principal component analysis showed similar results to the grouping results from the cluster analysis. The relative position of each accession in each group is influenced by the role of each character in forming a group. In addition, the diagram shows the role of each character indicated by different arrow lengths. If the arrow is getting longer, then it shows the character's role that is getting higher in the accession grouping. In general, results of the principal component analysis of 63 morphological characters divided 39 *Citrus* accessions into 2 main groups which were strongly influenced by tree shape, spine surface, leaf blade shape, fruit base shape, oil gland surface, presence of areola, and cross-section of the fruit axis.

In conclusion, the genus *Citrus* in the coastal area of Tomini Bay has unique and diverse morphological characters that are influenced by genetic and environmental factors. The diversity index value is 0.8103 which means low diversity. The grouping of 39 *Citrus* accessions on the dendrogram produces a similarity coefficient of 64.3% which forms 2 main clusters which are categorized into minor category classification at the species level diversity level. Furthermore, information on *Citrus* diversity can become the basis for germplasm conservation and plant breeding.

ACKNOWLEDGEMENTS

We thank the Directorate of Research, Technology, and Community Service, Ministry of Education, Culture, Research, and Technology, Indonesia, for providing research fund through the Research Program Fund for Fiscal Year 2022 the second phase, SKIM for Basic Research for Higher Education (PDUPT) with contract no. 225/E5/PG.02.00.PT/2022 with SK Rector no. 478/UN47/HK.02/2022.

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