



Discrimination between the cultivated species *Passiflora edulis* and the new species *Passiflora cacao* using *trnL* intron sequence, morphology and reproductive system

Américo José Carvalho Viana¹ · Margarete Magalhães Souza¹ · Juliane dos Santos Amorim¹ · Ronan Xavier Corrêa¹ · Ioná Santos Araújo Holanda² · Dário Ahnert¹ · Léonard Jean Victor Nicolas¹

Received: 27 May 2025 / Accepted: 4 February 2026 / Published online: 16 March 2026
© The Author(s) 2026

Abstract

The genus *Passiflora* L. is the most important in family *Passifloraceae*, with more than 520 species. However, new species have been discovered and characterized. Many of these species are used as food, medicine, cosmetics and ornamentation. The cultivated passion fruit *P. edulis* Sims, also known as purple passion fruit tree, occurs in the Atlantic Forest of Southern Bahia state, Brazil. Another species was recently described, *P. cacao*, which is morphologically very similar to *P. edulis*. Their *trnL* intron sequence, morphology and reproductive system were characterized to better differentiate these two species. According to the comparison of the *trnL* intron sequence among species of the subgenus *Passiflora*, the *P. cacao* species was in a more basal position and more distant from *P. edulis* than the other species of this subgenus. Based on morphological descriptors for leaf, flower, fruit and pollen data, it was possible to structure the diversity found in *P. cacao* and *P. edulis*. Two distinct groups were formed, represented by the accessions of each species. Regarding on the pollen/ovule ratio, both species were included in the category of facultative autogamous but crossability percentages classified the species as allogamous. *Passiflora edulis* and *P. cacao* showed good crossbreeding rates, indicating that they are closely related species. Based on the comparison of the *trnL* intron sequence, pollen characteristics and vegetative morphology, *P. cacao* was included in the subgenus *Passiflora* group. Analyses based on the *trnL* intron sequence were effective in distinguishing between the two species and confirming the character of the individual genotype.

Keywords CpDNA · Morphological characterization · Palynology · *Passiflora* · Passionflower

✉ Margarete Magalhães Souza
souzamagg@yahoo.com.br

Américo José Carvalho Viana
ameriviana@gmail.com

Juliane dos Santos Amorim
juliane_jsa@hotmail.com

Ronan Xavier Corrêa
ronanxc@uesc.br

Ioná Santos Araújo Holanda
iona@ufersa.edu.br

Dário Ahnert
darioa@uesc.br

Léonard Jean Victor Nicolas
lnicolas57@yahoo.fr

¹ Laboratório de Melhoramento de Plantas, Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz (UESC), Rod. Ilhéus-Itabuna, Km 16, Ilhéus 45662-900, Brazil

² Departamento de Ciências Agronômicas E Vegetais, Universidade Federal Rural Do Semi-Árido (UFERSA), Mossoró, Brazil

Introduction

The *Passifloraceae* family A. L. de Jussieu ex Kunth (Malpighiales Jussieu order; Milward-de-Azevedo and Baumgratz 2004), with more than 650 species distributed across 17 genera (Bernacci et al. 2013), is predominantly found in the tropical and subtropical areas of the Americas and Africa (Cervi and Imig 2013) and in the neotropical regions, especially Brazil (Bernacci et al. 2013). The family is known worldwide for its ornamental value, mainly attributed to its exotic flowers of varying colors and sizes, with an abundant number of flowers and flowering season more than once a year (Vanderplank 2000; Ulmer and MacDougal 2004; Abreu et al. 2009).

The genus *Passiflora* L., commonly referred to as passionfruit, is the most significant genus within the family *Passifloraceae*, containing more than 520 species (Cervi and Imig 2013). Recent discoveries have led to the identification and characterization of new species (Milward-De-Azevedo 2019; Mezzonato-Pires et al. 2020). Brazil is home to more than 130 native species, primarily found in the central and northern parts of the country (Bernacci et al. 2015). These species serve diverse purposes, such as in food (Ramaiya et al. 2019), medicine (He et al. 2020), cosmetics (Yanasan et al. 2024) and ornamental plants (Abreu et al. 2009; Nóbrega et al. 2017). The cultivated passionfruit exists in two main botanical forms: one with purple fruit, known as *P. edulis* Sims, and another with yellowish fruit, referred to as *P. edulis* f. *flavicarpa* O. Deg. Additionally, there are cultivars that produce fruits with colours that fall between these two forms (Jesus et al. 2017).

Due to the extensive size of the *Passiflora* genus, there are variations in the taxonomy of its species. Most taxonomic research on *Passiflora* relies on the morphological characteristics of the plants, resulting in classifications that encompass species and varieties. However, closely related species often exhibit morphological differences that are challenging to observe and characterize, necessitating molecular biology techniques or other techniques for more precise differentiation between these groups (Viana et al. 2010; Viana and Souza 2012). *Passiflora cacao* Bernacci and M.M. Souza have often been mistaken for *P. iodicarpa* Barb. Rodr. because of their similar morphological features described in the literature. However, *P. iodicarpa* is not observed in its natural habitat and is now regarded as a synonym of *P. edulis*, mainly due to poor reports of collection and herborized material for taxonomic studies (Bernacci et al. 2008; Bernacci and Souza 2012).

The chloroplast DNA (cpDNA) of plants are highly conserved in relation to gene structures and content (Efe et al. 2025) and has a slow substitution rate compared to the

nuclear genome (Li et al. 2023). The angiosperm cpDNA genome ranges from 107 to 218 kb and is organized as a circular DNA molecule with a highly conserved tetragonal structure (Sevindik et al. 2024). Some characteristics of the chloroplast genomes, like simple circular structure, clonal inheritance predominantly along the maternal line, and high variability (Tang et al. 2022) make it a great option for analysis of phylogenetic relationships between species (Efe et al. 2025), even at low taxonomic levels (Tang et al. 2022).

Among the molecular biology methods used to study genetic divergence between species, the cpDNA method has been frequently applied in taxonomic and phylogenetic research (Efe et al. 2025). This is due to its high level of conservation, its modes of inheritance (whether paternal, maternal, or biparental), as well as its low mutation rate (Tynkevicha et al. 2023). In phylogenetic studies, genes responsible for the transcription of tRNAs for tyrosine (*trnT*), leucine (*trnL*), and phenylalanine (*trnF*) are among the most frequently utilized regions of chloroplast DNA. The phylogenetic relationships within the genus *Passiflora* were reconstructed using *trnT*-*L*-*F* sequences and *trnL* intron sequences (Pádua 2005). The analysis included 64 species, which were categorized into four main clades: *Decaloba*, *Deidamioides*, *Astropheae*, and *Passiflora*. These clades align with the four subgenera identified by MacDougal and Feuillet (2004).

Morphological traits, also called descriptors, can offer a concise representation of the genetic relationships between populations and accessions (Castillo et al. 2020). To assess the discriminatory potential of these traits, it is essential to evaluate their overall contribution through multivariate analysis (Cruz et al. 2012).

The incidence of *Passiflora* species in fragments of the Atlantic Forest of Brazil includes many species that are considered native (Bernacci et al. 2015). Some of these wild species exhibit ornamental characteristics (Santos et al. 2012; Nóbrega et al. 2017; Souza et al. 2020), while others possess genes that confer resistance to diseases affecting cultivated passion fruit vine (Pereira et al. 2019; Santos et al. 2019). However, deforestation has severely impacted tropical regions, and the shrinkage of forest fragments has significantly altered the landscape (Rezende et al. 2018; Bourgoïn et al. 2021).

Like other *Passiflora* species, *P. edulis* and *P. cacao* share several morphological traits, necessitating studies to clarify their distinctions. To avoid confusing *P. cacao* with the wild form of *P. edulis*, it is essential to assess the genetic diversity between the two species. This study aimed to determine the relationship between *P. edulis* and *P. cacao* by examining the *trnL* intron sequence, as well as morphological descriptors and reproductive studies.

Material and methods

Study species and site description

The plant material consisted of five accessions of *Passiflora edulis* Sims and five accessions of *P. cacao* Bernacci and M.M. Souza (ex *P. cacaensis*; Viana and Souza 2012). The accessions of *P. edulis* were seeds donated by the Universidade Estadual do Norte Fluminense Darcy Ribeiro – UENF, Campos dos Goytacazes, RJ, Brazil (accessions BGP349 and BGP350), Empresa Brasileira de Pesquisa Agropecuária – EMBRAPA Cerrados, Planaltina, D.F., Brazil (accessions BGP351 and BGP352) and Instituto Agrônomo – IAC, Campinas, SP, Brazil (accession BGP353). *P. cacao* accessions (BGP344–348) were collected in the fragment of the Atlantic Forest of Southern Bahia State, at Morro da Viúva, Reserva Particular do Patrimônio Natural (RPPN) from Serra Bonita (Uiraçu Institute; www.uiraçu.org.br), Camacan, BA, Brazil, 14°47'S, 39°02'W, 789 m a.s.l (Fig. 1). Plants were grown in a greenhouse at the Work Collection of *Passiflora*, located between coordinates 14°39'S, 39°10'W; 78 m a.s.l.

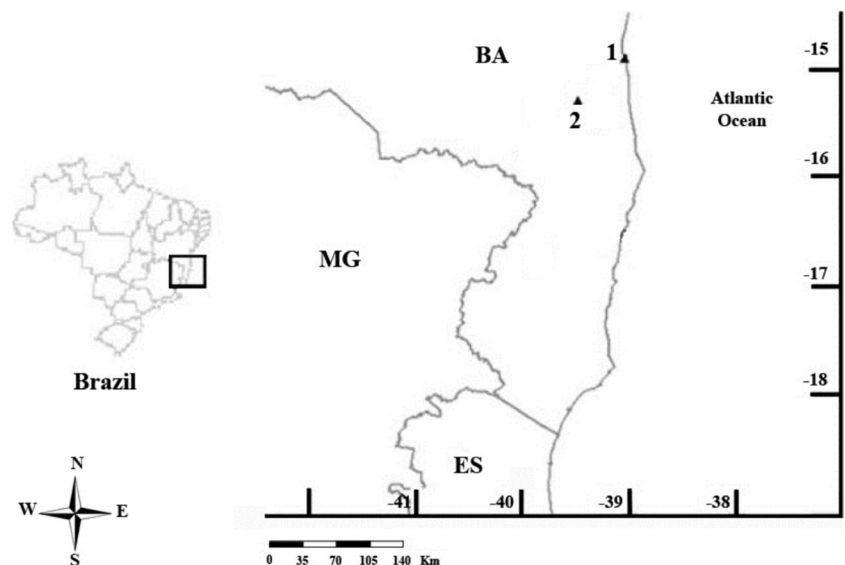
trnL cpDNA sequence analysis

Leaves were collected in bulk to extract chloroplastic DNA from five genotypes of *P. cacao*. DNA was extracted as described by Doyle and Doyle (1990), amplified in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, USA) using the primers *trnL*-c (CGAAATCGG TAGACGCTACG) and *trnL*-d (GGGGATAGAGGGACT TGAAC) developed by Taberlet et al. (1991). The fragments were amplified according to Gielly and Taberlet (1994) and

followed the program: 94 °C for 5 min + 35 cycles of 94 °C at 1 min, 50 °C at 1 min, 72 °C for 1 min + 72 °C for 7 min. The purified products deriving from amplifications were sequenced using the DYEnamic™ ET Terminator Sequencing Premix sequencing kit in the MegaBace DNA Analysis System 1.000 sequencer (MegaBace™) (Amersham Pharmacia Biotech. Inc, Buckinghamshire, UK).

After obtaining the *trnL* intron sequences of *P. cacao*, the GenBank database of the National Center for Biotechnology Information (NCBI) was searched to obtain the *trnL* intron sequences of other *Passiflora* species. The species used for the analysis and their respective accession numbers in the GenBank were: *P. edulis* – AF454783 and *P. alata* – AF454778 (Ossowski and Hunter, unpubl.); *P. coriacea* – DQ238789, *P. cincinnata* – DQ238792, *P. foetida* – DQ238796, *P. incarnata* – DQ238793, *P. subanceolata* (ex *P. palmeri*) – DQ123056, *P. sprucei* – DQ123096, *P. xiikzodz* – DQ238790 (Muschner et al. 2006) and *Clusia major* – AY144060 (Hale et al. 2004). The species *P. coriacea* and *P. xiikzodz* were included as material for external comparison (outgroup) of the subgenus *Passiflora* and the species *Clusia major* was included as material for external comparison (outgroup) of the family *Passifloraceae*. The consensus sequence of *P. cacao* was the result of analysis of six sequences, three originated from primer forward and the other three originated from reverse. The *trnL* intron sequence of *P. cacao* was aligned with the *trnL* intron sequences of other species, using the ClustalW program, which was implemented in the MEGA (*Molecular Evolutionary Genetics Analysis*) program, version 5.05 (Tamura et al. 2011). The cladogram was constructed using the Maximum Likelihood method (Felsenstein 1981), based on the Jukes-Cantor model (Jukes and Cantor 1969). The percentage of trees on which the associated taxa clustered together

Fig. 1 Map of the collection and maintenance site of the species studied. Location for species maintenance at the Active Germplasm Bank of *Passiflora* located at the campus of Universidade Estadual de Santa Cruz (UESC), Ilhéus, Bahia State, Brazil (1) and collection site of *P. cacao*, at the natural reserve Reserva Particular do Patrimônio Natural, Serra Bonita, Camacan, Bahia State, Brazil (2)



was calculated for 1000 repetitions and is shown next to the branches. Based on the alignment, a matrix of genetic distances between accessions was generated, calculated according to the Tamura-Nei replacement model, with ambiguous positions treated as pairwise deletion (Kumar et al. 2018).

Morphological characterization

Data on the morphological characterization of leaf, flower, fruit and pollen were obtained from five replicates per accession, thus totalling 25 replicates per species. Morphological characterization consisted of 22 descriptors, according to the *Serviço Nacional de Proteção de Cultivares do Ministério da Agricultura e Pecuária* (National Service for the Protection of Cultivars at the Ministry of Agriculture and Livestock) (MAPA 2008), comprising leaf, flower, fruit and pollen. The descriptors analysed for leaf, flower and fruit were: trilobate leaf width (TLW), trilobate leaf length (TLL), entire leaf width (ELW), entire leaf length (ELL), trilobate leaf area (TLA) and entire leaf area (ELA) in cm²; flower diameter (FD); fruit equatorial diameter (ED), fruit longitudinal diameter (LD) in mm; fruit weight (FW) in g; and fruit conformity index (CI) obtained by the relation between the two diameters of the fruit. For the descriptors analysed for pollen, terminologies were adopted according to Barth and Melhem (1988) and Punt et al. (2007) and were polar axis (PA), equatorial diameter (ED), colpus width (CW), mesocolpium (ME), polar view (PV), apocolpium (AP), Murus (MU), lumen (LU), Brochus (BR) in nm; polar area index (PAI) and polar axis/equatorial diameter ratio (P/E). Leaf area was obtained using a LI-3100 automatic meter (Li-Cor, Nebraska, USA), width, length and diameter measurements were obtained using a digital caliper rule. The pollen measurements were obtained from scanning electron microscope photomicrographs at the Universidade Estadual de Feira de Santana (UEFS/BA, Brazil). Pollen grains were fixed in FAA 1:1:1 (formalin, alcohol, and glacial acetic acid), deposited on stubs, dried in vacuum chambers, covered with a thin layer of gold dust, and observed through a LEO 1430 VP scanning electron microscope. Twenty-five measurements of each pollen characters per species were assessed. During pollen and morphological analysis, *P. rubra* was incorporated as a material for internal comparison to the genus.

Reproductive system characterization

Pollinations were performed since the flower opening time at 1 h intervals, with four repetitions (flowers) per time in each accession. Intraspecific hybridizations were carried out at 12 times (7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18 h) for each accession of *P. cacao*, totalling 240 flowers analysed, and at seven times (12, 13, 14, 15, 16, 17 and

18 h) for each accession of *P. edulis*, totalling 140 flowers analysed. Interspecific hybridizations were performed at the same seven times and in a single direction, whereas *P. cacao* was the pollen receptor vs. *P. edulis* as pollen donor, thus totalling 140 flowers analysed. The fertilization percentage was assessed five days after pollination, considering that flowers were fertilized once the fruit started developing. To the pollen-ovule ratio analysis, 10 flowers of each species, were used to count the number of pollen grains per anther and the number of ovules per ovary (Dafni 1992). The P:O ratio was used as an indicator of the preferred mode of reproduction of the plant, being: Cleistogamy (< 5.4); obligatory autogamy (5.5 to 39); facultative autogamy (39.1 to 396.9); facultative allogamy (397–2588) and obligatory allogamy (> 2588) (Cruden 1977).

Statistical analysis

All the statistical analysis of the data on the morphological characterization were performed considering a completely randomized experimental design. Statistical computations were conducted for descriptor analysis using GENES version 2007.0 (Cruz 2006) and R version 2.7.2 (R Development Core Team 2008) softwares. Descriptors data were submitted to analysis of variance (ANOVA) and the means were compared using the Scott-Knott test at 5% probability. For multivariate analysis, the Mahalanobis genetic distances were calculated on the variables to construct a dendrogram could be constructed based on the Unweighted Pair-Group Method using Arithmetic Averages (UPGMA). The relative contribution of each characteristic to the genetic diversity among species was based on the criteria proposed by Singh (1981). The mean values of *P. rubra* (Amorim et al. 2011) were included as an external group for comparison. The reliability of the cophenetic matrix was calculated by the cophenetic correlation coefficient (r_{cop}). For data analysis of self-pollination in *P. edulis* and interspecific pollinations, statistical computations were conducted using the GENES version 2007.0 (Cruz 2006) software. The pollination times were considered treatments and the effects of pollination times on the fertilization of flowers were studied using regression analysis. ANOVA was performed as for number of pollen grains, number of ovules and pollen-ovule ratio; the species were considered as treatments, comparing the accessions.

Results

trnL cpDNA sequence analysis

The *trnL* intron sequence of *P. cacao* was deposited in the GenBank [GenBank: HM212772], with total length of 502

base pairs (bp), while this intron had 605 bp in *P. edulis*. (Fig. 2). From the alignment of the sequences of the *P. cacao*, *P. edulis*, *P. alata* Dryand., *P. cincinnata* Mast., *P. foetida* var. *foetida* L., *P. sublanceolata* (Killip) MacDougal (ex *P. palmeri* Rose var. *sublanceolata* Killip), *P. incarnata*, *P. sprucei* Mast., *P. coriacea* Juss and *P. xiikzodz* J.M. MacDougal species, 32 (4.8%) parsimoniously informative sites, 494 (73.6%) conserved, 128 (19.0%) variable and 93 (13.8%) singleton (only two different nucleotides) sites were

detected. The percentages of singletons and parsimoniously informative regions were included in the class of variable sites; thereby, the total of percentages did not result in 100%. The estimates of the percentage of genetic distances between sequences are presented in Table 1.

In the cladogram based on the Jukes-Cantor model, by the maximum likelihood method from the *trnL* intron sequence (Fig. 3), it was noted that *P. coriacea* and *P. xiikzodz*, which belonged to the subgenus *Decaloba*, were in a more basal

Fig. 2 A *trnL* gene partial sequence of *Passiflora cacao* – chloroplast gene for chloroplast product

```
AAATTGGATTGAGCCTTGGTATGGAACTTACTAAGTTGGATAACTTTCAAATTCAGAGAAA
CCCTGGAATAATAAAATGGGCAATCCTGAGCCAAATCCTGTTTTTCGAAAACAAAAAACA
ACAAAGGTTTCATAAAGACAGAATCAGAATAAATAAAGGATAGGTGCAGAGACTCAACGGA
AGCTGTTCTAACAAATGGAGTTGATTGCGTTGCATAAAGGAATCCTTTCTATTAATAAATCCA
GAAAAGATAAAGTGATAAAATAAAGATAACACTAATATACATAGATCCACGTACTGAAAT
ACTATTTCAAATAAAATAATTAATGACGAGTGACCCAAACAGTATCTATATTTTTCTGT
ATCTTTTTTTTTTCATTTTTTATGGAACAAGTTGTGTGAATCAAACTTCTAAGTTGAAGT
AAGAATCGAATTAATTGGTCAAATCAATTGATCAAATAAATAACGTACTCCATAATCTGA
TAGATAACG
```

Table 1 Estimates of the percentage of genetic distances between sequences for characterization of *Passiflora edulis* and *P. cacao*

Species	<i>P. alata</i>	<i>P. cincinnata</i>	<i>P. edulis</i>	<i>P. foetida</i>	<i>P. incarnata</i>	<i>P. sublanceolata</i>	<i>P. cacao</i>	<i>P. sprucei</i>
<i>P. alata</i>	0							
<i>P. cincinnata</i>	0.547	0						
<i>P. edulis</i>	0.182	0.365	0					
<i>P. foetida</i>	0.914	1.097	0.731	0				
<i>P. incarnata</i>	0.365	0.548	0.182	0.916	0			
<i>P. sublanceolata</i>	0.952	1.145	0.762	0.000	0.954	0		
<i>P. cacao</i>	3.036	3.245	2.834	3.659	3.043	3.586	0	
<i>P. sprucei</i>	0.762	0.954	0.571	1.336	0.763	1.331	2.954	0

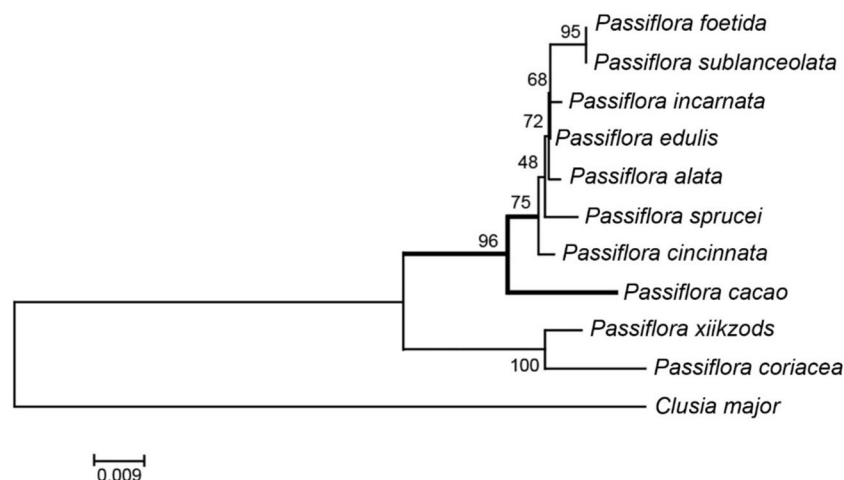


Fig. 3 Cladogram representing the phylogenetic relationships among *Passiflora* species using the *trnL* intron sequence. The cladogram was constructed using the Maximum Likelihood method based on the Jukes-Cantor model. The percentage of trees on which the associated taxa clustered together is shown next to the branches. The species *P.*

coriacea and *P. xiikzodz* were included as outgroup of the subgenus *Passiflora* and the species *Clusia major* was included as outgroup of the family *Passifloraceae*. The species *P. edulis*, *P. cacao*, *P. alata*, *P. cincinnata*, *P. foetida*, *P. incarnata*, *P. sublanceolata* and *P. sprucei* are the ingrouping of the subgenus *Passiflora*

position concerning other species of the subgenus *Passiflora*. Among the species of the latter subgenus, *P. cacao* was in a more basal position and more distant from *P. edulis*, than the other species of this subgenus.

Based on comparison of the *trnL* intron sequence, observed a clear separation between the genotypes *P. cacao* and *P. edulis*. Also, according to the comparison of the *trnL* intron sequence, *P. cacao* was included in the subgenus *Passiflora* group.

Morphological characterization

Passiflora edulis showed mean values for the morphological characteristics of leaf, flower and fruit higher than those of *P. cacao* in most the variables, except for ELL (Table 2). Concerning most variables, the F test ($P < 0.05$) showed significant differences between the two species, except for TLL, ELW and CI (Table 3). The FW characteristic showed the greatest difference between the two species, with an average weight of 184.8 and 8.7 g for *P. edulis* and *P. cacao*, respectively. As for the relative contribution of each characteristic to the genetic diversity among species, it was verified among the descriptors measured in leaf, flower and fruit, that the ones most contributing to diversity were FW, with 67.9%, followed by ED, with 16.3%, and FD, with 11.7%.

The main differences observed in pollen grain were noted in the ornamentations. Pollen grains of both species were classified as large, with mean value of 55.1 μm , subspheroidal shape (oblate spheroidal), isopolar, on average with small polar area of 22.2 μm , syncolpate, and reticulate exine. The three pairs of colpi are joined by their edges thereby forming a ring around the mesocolpium (Fig. 4). The six mesocolpia observed are wider between the colpi of a same pair than between pairs of adjacent colpi. The muri in *P. edulis* are smooth and sinuous, while the lumen presents some bacula, and two brochi are found parallelly aligned between two pairs of colpi. The muri in *P. cacao* are furrowed and with several pila. In this species, the lumen shows a large quantity of bacula. There is only one row of brochus between two pairs of colpi. The average pollen descriptor data observed in *P. edulis* were higher for most characteristics, except for AP, PAI and LU (Table 4). For most variables, the F test ($P < 0.05$) showed significant differences between the two species, except for CW and LU (Table 5).

We observed the formation of three well-defined groups in the dendrogram (Fig. 5), in the cluster analysis with morphological descriptors for leaf, flower, fruit and pollen. The first, second and third groups corresponded to the accessions of *P. edulis*, *P. cacao* and *P. rubra*, respectively. Besides being effective in correctly separating the accessions of each species, this grouping still showed a high cophenetic correlation coefficient ($r_{\text{cof}} = 0.97$), which promoted confidence within the clustering.

Table 2 Mean values and standard deviation of morphological traits for characterization of *Passiflora edulis* and *P. cacao*

Species	Access	TLW (mm)	TLL (mm)	TLA (cm ²)	ELW (mm)	ELL (mm)	ELA (cm ²)	FD (mm)	ED (mm)	LD (mm)	CI	FW (g)
<i>P. edulis</i>	349	124.5 ± 08 a	139.3 ± 13 a	109.0 ± 18 a	72.6 ± 05 a	116.2 ± 08 b	105.9 ± 8 a	71.2 ± 4 a	71.5 ± 6 a	90.3 ± 3 a	1.3 ± 0 a	180.8 ± 10 b
	350	128.3 ± 10 a	143.1 ± 07 a	114.6 ± 17 a	69.4 ± 07 a	102.7 ± 12 b	61.9 ± 6 c	70.5 ± 2 a	70.4 ± 6 a	88.2 ± 5 a	1.3 ± 0 a	177.4 ± 13 b
	351	132.1 ± 10 a	147.9 ± 07 a	111.0 ± 15 a	73.0 ± 04 a	109.1 ± 17 b	77.9 ± 6 b	71.9 ± 2 a	66.5 ± 1 b	85.0 ± 4 b	1.3 ± 0 a	179.4 ± 13 b
	352	135.6 ± 08 a	149.1 ± 05 a	81.4 ± 10 b	72.2 ± 10 a	102.5 ± 19 b	72.8 ± 3 b	69.5 ± 1 a	73.3 ± 6 a	89.7 ± 2 a	1.2 ± 0 a	196.2 ± 01 a
	353	116.2 ± 10 b	126.2 ± 06 b	111.2 ± 14 a	74.4 ± 07 a	108.8 ± 06 b	68.7 ± 1 b	70.6 ± 3 a	66.7 ± 2 b	86.9 ± 3 b	1.3 ± 0 a	190.4 ± 05 a
<i>P. cacao</i>	M	127.3 ± 10 a	141.1 ± 11 a	105.5 ± 18 a	72.3 ± 06 a	107.9 ± 13 b	77.5 ± 4 b	70.7 ± 2 a	69.7 ± 4 a	88.0 ± 3 a	1.3 ± 0 a	184.8 ± 11 a
	344	110.9 ± 06 b	142.5 ± 08 a	78.2 ± 13 b	69.3 ± 09 a	126.2 ± 06 a	51.8 ± 08 c	34.5 ± 4 b	36.1 ± 1 c	45.4 ± 1 c	1.3 ± 0 a	7.4 ± 0 c
	345	112.8 ± 08 b	145.6 ± 09 a	101.9 ± 24 a	72.0 ± 07 a	129.6 ± 13 a	50.4 ± 07 c	37.2 ± 1 b	35.3 ± 3 c	45.2 ± 1 c	1.3 ± 0 a	9.8 ± 1 c
	346	97.7 ± 14 c	126.0 ± 11 b	68.5 ± 13 b	68.7 ± 09 a	129.4 ± 05 a	72.8 ± 24 b	38.7 ± 1 b	34.8 ± 2 c	45.1 ± 1 c	1.3 ± 0 a	7.8 ± 2 c
	347	129.5 ± 21 a	146.6 ± 19 a	90.1 ± 05 b	68.4 ± 09 a	130.9 ± 13 a	67.4 ± 15 b	35.1 ± 5 b	34.9 ± 1 c	45.5 ± 1 c	1.3 ± 0 a	9.5 ± 2 c
<i>P. cacao</i>	348	93.3 ± 07 c	118.5 ± 13 b	80.1 ± 07 b	69.1 ± 10 a	127.5 ± 06 a	55.5 ± 18 c	34.6 ± 5 b	35.6 ± 2 c	43.6 ± 3 c	1.2 ± 0 a	9.0 ± 1 c
	M	108.9 ± 17 b	135.8 ± 16 a	83.8 ± 17 b	69.5 ± 08 a	128.7 ± 08 a	59.6 ± 16 b	36.0 ± 3 b	35.3 ± 1 c	44.9 ± 1 c	1.3 ± 0 a	8.7 ± 2 c

Values in the same column followed by the same letter are not significantly different according to the Scott & Knott test at 5% probability level.

Access Accession means, M Species mean, TLW Trilobate leaf width, TLL Trilobate leaf length, TLA Trilobate leaf area, ELW Entire leaf width, ELL Entire leaf length, ELA Entire leaf area, FD Flower diameter, ED Fruit equatorial diameter, LD Fruit longitudinal diameter, CI Fruit conformity index, FW Fruit weight

Table 3 ANOVA summary for morphological traits between *Passiflora edulis* and *P. cacao*

SV	FD	Mean squares										
		TLW	TLL	TLA	ELW	ELL	ELA	FD	ED	LD	CI	FW
Accessions	9	1051.2*	592.9 ^{NS}	1420.4*	23.1 ^{NS}	684.0*	1301.5*	1682.2*	1660.1*	2587.7*	0.0 ^{NS}	43,226.5*
Error	40	121.6	113.2	216.5	60.1	130.5	136.1	10.0	11.8	7.2	0.0	47.2
CV (%)		9.3	7.6	15.0	10.0	9.6	17.2	5.9	6.5	4.0	6.6	7.1

* Significant at 5% ($P < 0.05$) of probability by F test. ^{NS} Not Significant

SV Source of variation, FD freedom degrees, CV Coefficient of variation, TLW Trilobate leaf width, TLL Trilobate leaf length, TLA Trilobate leaf area, ELW Entire leaf width, ELL Entire leaf length, ELA Entire leaf area, FD Flower diameter, ED Fruit equatorial diameter, LD Fruit longitudinal diameter, CI Fruit conformity index, FW Fruit weight

Reproductive system characterization

After self-pollination, *P. edulis* showed an average of 24.5% fertilized flowers at the opening, which decreased to 11.4% at the last pollination, while no flower was fertilized during the self-pollination times of *P. cacao* (Table 6). As for the reproductive capacity evaluated by means of interspecific hybridization, 59.3% fertilization was observed in pollinated flowers at the opening and a decrease to 45.5% at the last pollination (Table 6). There was a significant difference by the F test ($P < 0.05$) between the pollination times tested. The fertilization percentage was negatively influenced by the last pollination times (Fig. 6). There was a linear relationship between the percentage of fertilized flowers (Y) and the pollination times (X). Furthermore, the percentages of fertilized flowers declined according to the duration of flower opening in all the accessions. *P. edulis* showed an average of 56,194 pollen grains per anther and 284 ovules per ovary, whereas *P. cacao* showed an average of 10714 pollen grains per anther and 113 ovules per ovary. There was a significant difference by the F test ($P < 0.01$) between species, for the number of pollen grains and ovules, and the pollen-ovule ratio, which showed mean values of 197.8 and 94.7 for *P. edulis* and *P. cacao*, respectively. Both species were included in the category of facultative autogamous considering the P:O ratio.

Discussion

trnL cpDNA sequence

The nucleotide *trnL* intron sequence of *Passiflora* species is notably conserved (Muschner et al. 2003, 2012; Hansen et al. 2006; Krosnick et al. 2013), which accounts for the low percentage (4.8%) of variable sites providing parsimonious data. In *P. cacao* and *P. edulis*, the GC content was found to be 30.2% and 31.9%, respectively, aligning with the 29% average observed by Hansen et al. (2006) when comparing *trnL*-T spacer sequences across 57 *Passiflora* species. This

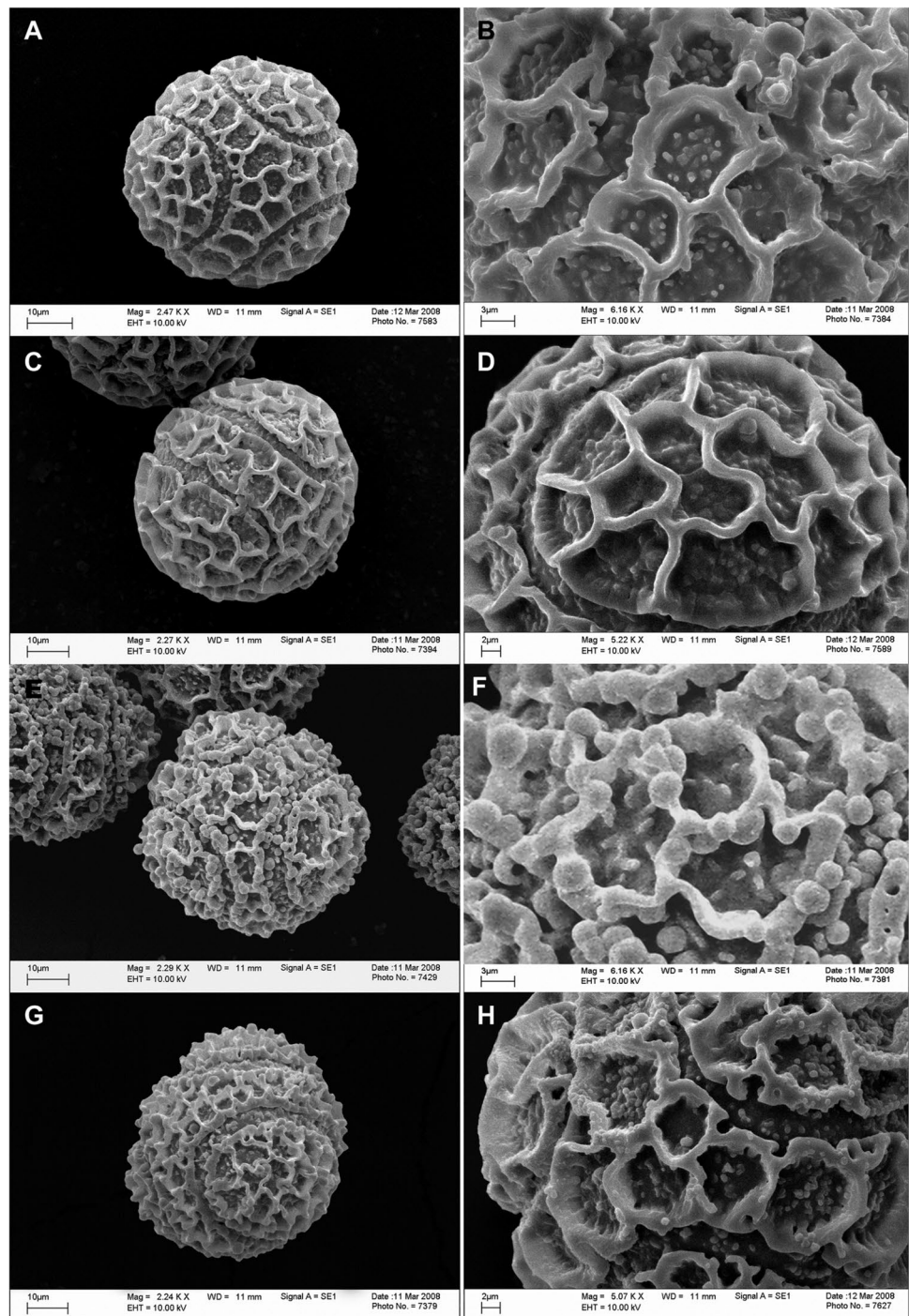
relatively low GC content in the non-coding regions of the *trn* gene is a common characteristic within the genus *Passiflora* (Souza et al. 2004a; Yotoko et al. 2011; Santos et al. 2014).

When examining the cladogram representation of species from the subgenera *Passiflora* and *Decaloba*, it became apparent that the divergences between the subgenera were more significant than those observed among species within each subgenus. In this cladogram, species belonging to the subgenus *Decaloba* were positioned more basally than those from the subgenus *Passiflora*. Furthermore, it was identified that the most derived characteristics of species in the subgenus *Passiflora* were based on non-coding sequences of the *trn* gene (Hansen et al. 2006; Muschner et al. 2006). *P. cacao* was the species that showed the greatest divergence from the others by being positioned more basally within the subgenus *Passiflora*. This confirms previous analyses based on morphological data indicating that *P. cacao* and *P. edulis* are distinct yet closely related species).

Phylogenetic studies have widely utilized analyses based on cpDNA sequences due to their structural stability, patterns of inheritance, and mutation rates (Morton 2002; Huang et al. 2014). Phylogenetic studies using cpDNA sequences have been crucial in understanding evolutionary relationships among *Passiflora* species (Muschner et al. 2003; Cauz-Santos et al. 2020). This tool has also been used to carry out phylogenetic reconstructions of species from different families, including the integrifolia complex, which encompasses several taxa with floral traits very similar to those of the species *Petunia integrifolia* (Hook.) Schinz & Thell. The sequences of internal spacers derived from nuclear ribosomal DNA (*ITS1* and *ITS2*) along with two intergenic spacers from chloroplast DNA (*trnS-trnG* and *psbA-trnH*) were used to differentiate five distinct species. This resulted in the recognition of a genotype that was categorized as a new species, listed as *Petunia integrifolia* (Longo et al. 2014).

In *Paubrasilia echinata* (Lam.) Gagnon, H. C. Lima & G. P. Lewis, a group of specimens exhibiting various leaf morphological traits has been identified, leading

Fig. 4 Pollen morphology observed in scanning electron microscope. *P. edulis* (A–D) pollen in polar view (Bar = 10 μ m) (A), ornamentation detail (Bar = 3 μ m) (B), pollen in equatorial view (Bar = 10 μ m) (C) and ornamentation detail (Bar = 2 μ m) (D), *P. cacao* (E–H) pollen in polar view (Bar = 10 μ m) (E), ornamentation detail (Bar = 3 μ m) (F), pollen in equatorial view (Bar = 10 μ m) (G) and ornamentation detail (Bar = 2 μ m) (H). Arrows indicate pairs of colpi joined at the poles



researchers to propose the existence of a new species based on the *trnL* intron sequence (Juchum et al. 2008; Souza et al. 2021). Phylogenies of several other genera have been reconstructed from the cpDNA sequences, as in *Cycas taitungensis* C. F. Shen, K. D. Hill, C. H. Tsou & C. J. Chen (Wu et al. 2007; Wu and Chaw 2015) and *Cryptomeria japonica* (Thunb. ex L.) D. Don (Hirao et al. 2008). In this study, the *trnL* intron sequence was identified as the primary differentiator between *P. edulis* and

P. cacao, even more so when compared to other species within the subgenus *Passiflora*.

Morphological characterization

Morphological descriptors have proven effective in distinguishing between *Passiflora* species and the two botanical forms of *P. edulis* (Castillo et al. 2020). Leaf characteristics were slightly divergent between the *P. edulis* and *P. cacao*

Table 4 Mean values and standard deviation of pollen characteristics in *Passiflora edulis* and *P. cacao*

Species	Access	PA (μm)	ED (μm)	CW (μm)	ME (μm)	PV (μm)	AP (μm)	PAI	P/E	MU (μm)	LU (μm)	BR (μm)
<i>P. edulis</i>	349	52.9 $\pm$ 1 b	57.1 $\pm$ 1 a	16.7 $\pm$ 3 a	31.1 $\pm$ 2 b	56.1 $\pm$ 1 a	22.5 $\pm$ 2 b	0.3 $\pm$ 0 c	0.9 $\pm$ 0 b	1.3 $\pm$ 0 a	8.7 $\pm$ 2 b	11.9 $\pm$ 2 b
	350	52.2 $\pm$ 1 b	56.9 $\pm$ 1 a	19.1 $\pm$ 2 a	33.3 $\pm$ 1 b	55.9 $\pm$ 2 a	21.4 $\pm$ 2 b	0.3 $\pm$ 0 c	0.9 $\pm$ 0 b	1.3 $\pm$ 0 a	10.0 $\pm$ 2 a	12.3 $\pm$ 2 b
	351	51.1 $\pm$ 2 c	54.1 $\pm$ 1 b	17.1 $\pm$ 1 a	35.1 $\pm$ 3 a	57.4 $\pm$ 3 a	19.1 $\pm$ 2 c	0.2 $\pm$ 0 d	0.9 $\pm$ 0 a	1.2 $\pm$ 0 a	8.9 $\pm$ 1 b	11.6 $\pm$ 1 b
	352	56.2 $\pm$ 3 a	57.9 $\pm$ 3 a	17.3 $\pm$ 1 a	37.2 $\pm$ 3 a	59.1 $\pm$ 4 a	19.8 $\pm$ 2 c	0.2 $\pm$ 0 d	0.9 $\pm$ 0 a	1.3 $\pm$ 0 a	10.3 $\pm$ 1 a	19.9 $\pm$ 1 a
	353	52.9 $\pm$ 2 b	57.6 $\pm$ 2 a	18.6 $\pm$ 2 a	32.8 $\pm$ 1 b	56.8 $\pm$ 1 a	20.6 $\pm$ 2 c	0.3 $\pm$ 0 c	0.9 $\pm$ 0 b	1.3 $\pm$ 0 a	9.7 $\pm$ 1 a	12.4 $\pm$ 1 b
	M	52.8 $\pm$ 2 b	56.6 $\pm$ 2 a	18.4 $\pm$ 3 a	33.5 $\pm$ 4 b	56.9 $\pm$ 2 a	21.3 $\pm$ 2 b	0.4 $\pm$ 0 b	0.9 $\pm$ 0 a	1.3 $\pm$ 0 a	9.4 $\pm$ 1 a	13.4 $\pm$ 3 b
<i>P. cacao</i>	344	53.8 $\pm$ 2 b	56.0 $\pm$ 1 a	13.8 $\pm$ 8 a	31.3 $\pm$ 6 b	56.3 $\pm$ 2 a	23.1 $\pm$ 3 b	0.4 $\pm$ 0 b	0.9 $\pm$ 0 a	1.0 $\pm$ 0 b	8.7 $\pm$ 0 b	12.1 $\pm$ 1 b
	345	50.9 $\pm$ 1 c	54.7 $\pm$ 2 b	15.2 $\pm$ 4 a	31.0 $\pm$ 2 b	53.6 $\pm$ 2 b	25.9 $\pm$ 4 a	0.5 $\pm$ 0 a	0.9 $\pm$ 0 b	1.1 $\pm$ 0 b	10.8 $\pm$ 1 a	12.9 $\pm$ 1 b
	346	52.5 $\pm$ 3 b	57.4 $\pm$ 2 a	17.4 $\pm$ 3 a	32.3 $\pm$ 2 b	55.7 $\pm$ 1 a	22.0 $\pm$ 1 b	0.3 $\pm$ 0 c	0.9 $\pm$ 0 b	0.9 $\pm$ 0 b	8.6 $\pm$ 1 b	11.8 $\pm$ 1 b
	347	49.3 $\pm$ 2 c	55.9 $\pm$ 3 a	19.3 $\pm$ 5 a	30.9 $\pm$ 1 b	56.8 $\pm$ 1 a	28.6 $\pm$ 1 a	0.5 $\pm$ 0 a	0.8 $\pm$ 0 b	1.1 $\pm$ 0 b	10.6 $\pm$ 2 a	12.8 $\pm$ 2 b
	348	48.8 $\pm$ 1 c	53.2 $\pm$ 2 b	15.7 $\pm$ 4 a	31.1 $\pm$ 1 b	51.0 $\pm$ 2 b	18.9 $\pm$ 2 c	0.3 $\pm$ 0 c	0.9 $\pm$ 0 b	1.2 $\pm$ 0 a	9.9 $\pm$ 1 a	12.2 $\pm$ 1 b
	M	50.6 $\pm$ 3 c	55.0 $\pm$ 2 a	16.4 $\pm$ 4 a	31.1 $\pm$ 3 b	54.4 $\pm$ 2 b	23.3 $\pm$ 4 b	0.5 $\pm$ 0 a	0.9 $\pm$ 0 a	1.1 $\pm$ 0 b	9.6 $\pm$ 1 a	12.1 $\pm$ 1 b

Values in the same column followed by the same letter are not significantly different according to the Scott & Knott test at 5% probability level.
Access Accession mean, *M* Species mean, *PA* Polar axis, *ED* Equatorial diameter, *CW* Colpus width, *ME* Mesocolpium, *PV* Polar view, *AP* Apocolpium, *MU* Murus, *LU* Lumen, *BR* Brochus, *PAI* Polar area index, *P/E* Polar axis/equatorial diameter ratio

Table 5 ANOVA summary for pollen descriptors between *Passiflora edulis* and *P. cacao*

SV	FD	Mean squares										
		PA	ED	CW	ME	PV	AP	PAI	P/E	MU	LU	BR
Species	9	22.9*	13.1*	15.5 ^{NS}	22.4*	24.0*	47.7*	0.0*	0.0*	0.1*	3.5 ^{NS}	30.2*
Error	40	4.0	3.5	15.8	6.9	4.2	5.2	0.0	0.0	0.0	1.3	1.7
CV (%)		3.8	3.3	23.3	8.1	3.7	10.5	8.6	3.2	14.2	11.7	10.0

* Significant at 5% ($P < 0.05$) of probability by F test. ^{NS} Not Significant

SV Source of variation, *FD* Freedom degrees, *CV* Coefficient of variation, *PA* Polar axis, *ED* Equatorial diameter, *CW* Colpus width, *ME* Mesocolpium, *PV* Polar view, *AP* a Pocolpium, *MU* Murus, *LU* Lumen, *BR* Brochus, *PAI* Polar area index, *P/E* Polar axis/equatorial diameter ratio.

Fig. 5 Dendrogram of *P. edulis* and *P. cacao*, grouped according to the Mahalanobs distance by the UPGMA clustering method, based on the morphological descriptors. *P. rubra* was added as an external group for comparison

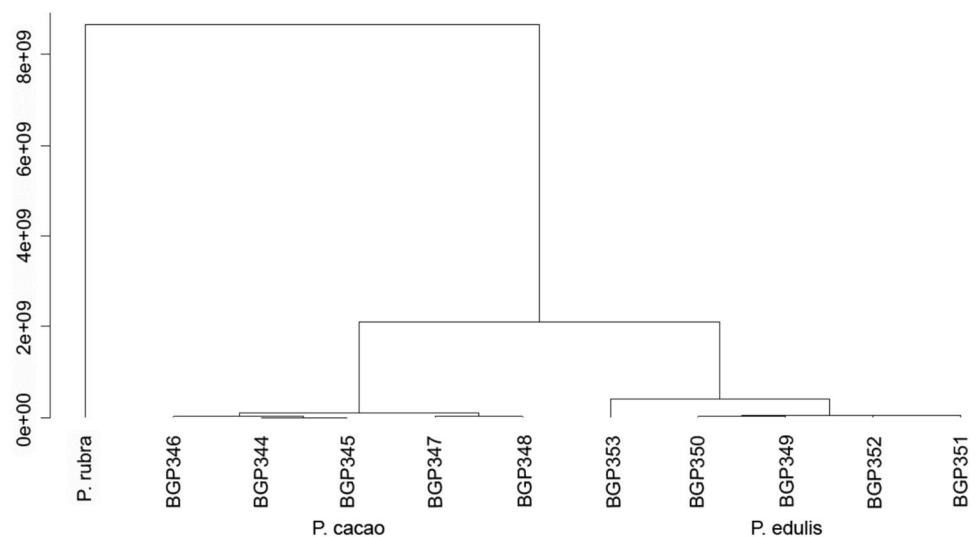


Table 6 Percentage of self-pollinations in *Passiflora edulis* and *P. cacao* and interspecific hybridizations

Pollination times (h)	Self-pollination <i>P. edulis</i>						Self-pollination <i>P. cacao</i>	<i>P. cacao</i> ♀ x <i>P. edulis</i> ♂					
	P 1	P 2	P 3	P 4	P 5	M		P 1	P 2	P 3	P 4	P 5	M
12:00	25.0	24.1	20.0	21.0	32.5	24.5	0.0	62.5	63.8	51.0	53.6	65.7	59.3
13:00	22.3	22.7	17.8	18.7	29.0	22.1	0.0	58.6	59.8	47.8	50.2	61.6	55.6
14:00	17.9	18.3	14.3	15.0	23.3	17.7	0.0	50.5	51.5	47.3	49.7	53.1	50.4
15:00	19.2	17.3	15.4	16.1	25.0	18.6	0.0	55.3	56.4	45.1	47.4	61.3	53.1
16:00	15.1	15.4	12.1	12.7	19.6	14.9	0.0	45.7	46.6	37.3	39.2	48.0	43.3
17:00	17.3	17.6	13.8	14.5	22.5	17.1	0.0	47.8	48.8	43.7	45.9	63.7	49.9
19:00	10.1	13.2	8.1	12.5	13.1	11.4	0.0	37.5	47.3	45.2	47.5	50.2	45.5

M Mean percentage, P Plant

leaf. Thus, there were no significant statistical differences for the TLL and ELW characteristics. Similar results were observed when three selections of purple passion fruit tree were phenotypically analysed (Meletti et al. 2005). No statistical difference concerning the length of leaf blade was observed among the accessions. In the characterization of

11 species, comprising eight accessions of *P. edulis* and 34 accessions of *P. edulis* f. *flavicarpa*, it was found that leaf descriptors, including leaf lobe length and total leaf width, did not provide significant differentiation between accessions of purple and yellow passion fruit trees (Crochemore et al. 2003). The two leaf types, entire and trilobed, found in *P. edulis* and *P. cacao* were examined independently because the entire leaf represents not only a transitional phase in the development of the trilobed leaf but also a distinct form exhibited by the two species (leaf dimorphism), which is characteristic of certain *Passiflora* (Imig et al. 2018). Leaf dimorphism, observed in *P. edulis* and *P. cacao*, is common among climbing plants and is an adaptation to different environmental conditions (Givnish and Vermeij 1976; Li et al. 2020).

The strong discriminatory capability demonstrated by the fruit descriptors in this research aligns with other findings in *P. edulis*, where four highly discriminating descriptors among 22 morphological descriptors were observed, of which two concerned the fruit (Crochemore et al. 2003). Accessions 'Paulista' and 'Maracujá-Maçã' of *P. edulis* had heavy, large, oval, and purple peeled fruit (Meletti et al. 2005) as found in the present study.

The average measurements recorded for *P. edulis* pollen specifically regarding the equatorial diameter, polar axis, colpus, and mesocolpium were in line with those found by Amela Garcia et al. (2002) and Araújo and Santos (2004), who studied natural species in Argentina and Chapada Diamantina in Bahia State, Brazil, respectively. The features of *Passiflora* pollen grains exhibited a consistent pattern of size and ornamentation specific to each subgenus, making them valuable for taxonomic research at both the subgeneric level (Milward-De-Azevedo et al. 2014; Araújo et al. 2019) and the species level. This led to the establishment of a clade for species identification based on pollen data (Milward-De-Azevedo et al. 2014; Mezzonato-Pires et al. 2017).

Based to the pollen classification (Barth and Melhem 1988), *P. cacao* is categorized under the subgenus *Passiflora* characterized by MacDougal and Feuillet (2004). As

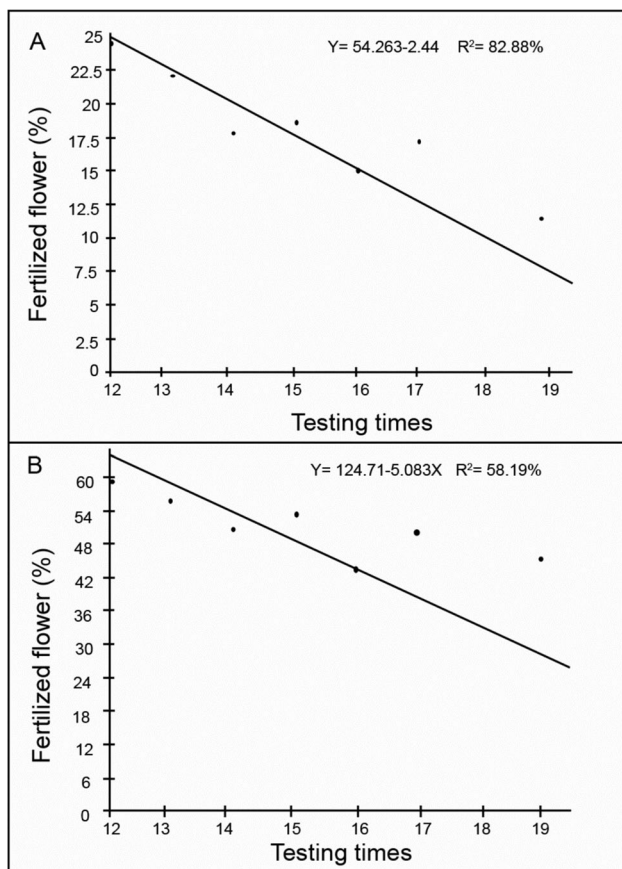


Fig. 6 Percentage of fertilized flowers in *Passiflora edulis* and *P. cacao* at seven pollination times, as of the flower opening time (12:00). Self-pollinated and fertilized flowers in *P. edulis* (A) and fertilized flowers from a hybridization between *P. cacao* and *P. edulis* (B)

per Spirlet's classification (Spirlet 1965), the pollen grains of *P. cacao* and *P. edulis* belong to the same type. Both species have type 1 pollen grains, characterized by a triradial pontopericulum and a reticulate exine (Araújo and Santos 2004). The intercolpal areas, including opercula, pontopercula, and pseudopercula, are commonly utilized in the palynological descriptions of species within the *Passifloraceae* family. However, the term "operculum" is appropriate for use only in exceptional instances (Erdtman 1943; Milward-De-Azevedo et al. 2010). The term describes areas that are slightly thickened and distinct on the apertural membrane. The operculum is a thickening of the exine that partially covers the opening, taking on circular, annular, elliptical, and pontoperculate forms (Thanikaimoni 1986). In this study, the term pontopericulum was utilized, following the definitions provided by Thanikaimoni (1986) and Punt et al. (2007). The pontopericulum is thus described as a band of exine that is fused at the apocolpus areas, which refers to the narrowest area (equatorial) located between two fused openings in relation to the polar area (Araújo and Santos 2004).

The differences in size, types of openings, and ornamentation of pollen grains within the *Passiflora* genus are correlated to the basic chromosome numbers described for this group (Hansen et al. 2006; Amorim et al. 2014). Species sharing the same basic chromosome number exhibited comparable patterns in their pollen characteristics (Amorim et al. 2014). Moreover, taxonomic classification based on pollen descriptors aligned more closely with the subgeneric classification of *Passiflora* as outlined by MacDougal and Feuillet (2004) than with that proposed by Killip (1938) (Liviston et al. 2005).

The cluster analysis involving morphological descriptors for leaves, flowers, fruits, and pollen demonstrated a clearer discrimination among *P. edulis* accessions compared to those from *P. cacao*. Expanding the set of descriptors utilized for characterizing an individual can also enhance the genetic analysis within the genome, as each phenotypic trait assessed by a descriptor reflects the expression of one or more genes in that individual, considering environmental influences as well. Therefore, employing a variety of variables leads to more reliable discrimination among accessions (Upadhyaya et al. 2012).

Reproductive system characterization

In interspecific hybridization between *P. cacao* and *P. edulis* was recorded a fertilization index above 50%, indicating a strong genetic relationship between these two species. *P. edulis* has demonstrated strong reproductive compatibility in crosses with genetically similar species, promoting the development of hybrids for introgression of genes for disease resistance or to transcribe for traits that improve the species' adaptation to climatic conditions (Santos et al. 2019).

Self-pollinations conducted on *P. edulis* at the time of flower opening yielded a low fertilization rate of less than 25%. Similar findings have been reported, indicating that this species exhibits 50% self-incompatibility (Madureira et al. 2014). In contrast, complete self-incompatibility (100%) was noted when flowers were pollinated after anthesis (Bruckner et al. 1995; Suassuna et al. 2003; Rêgo et al. 2013; Madureira et al. 2014), whereas 14.8% of flowers were successfully fertilized when pollinated on their floral buds, prior to anthesis (Bruckner et al. 1995). This suggests that self-fertilization is possible in *P. edulis*. Self-compatible plants of *P. edulis* have been reported, presenting corona coloration (completely white) as a morphological marker (Souza et al. 2010).

Early research on *P. edulis* indicated that self-incompatibility was governed by a single gene with three alleles: *S1*, *S2*, and *S3*, exhibiting complete dominance (Bruckner et al. 1995). Three new alleles of the *S* gene were found, from *S1* to *S6* (Rêgo et al. 1999). It was suggested the presence of another gene, the *G* gene, which has a gametophytic action and operates in conjunction with the *S* gene, which has a sporophytic action (Suassuna et al. 2003). Self-incompatibility in *P. edulis* arises not from a single gene, but rather from the interaction of a gene complex (Okamoto et al. 2007).

Both species were categorized as facultative autogamous based on the Cruden index (Cruden et al. 1977). However, the classification of the reproductive mode based on the ratio of pollen grain to ovule numbers was only applicable to *P. edulis*. In contrast, *P. cacao* exhibited complete self-incompatibility, making this classification unsuitable for that species. Discrepancies in the Cruden classification for various species have been noted. It was investigated the pollen/ovule ratio in both self-incompatible and self-compatible cacao cultivars (*Theobroma cacao* L.) and both types were labeled as obligatory autogamous; the TSH1188 cultivar, which shows self-incompatibility, demonstrates a deviation from the pattern proposed by Cruden (Godoy et al. 2009). Similarly, the reproductive mode of *Handroanthus pulcherrima* (Sandwith) S. O. Grose did not conform to the facultative xenogamy classification due to the observed self-incompatibility within this genus, suggesting that it behaves as obligatory xenogamic (Souza et al. 2004b). These results highlight that the Cruden index may not be suitable for many plant species, as seen in the case of *P. cacao*.

Both species exhibited a decline in fertilization rates following anthesis. In *P. edulis* f. *flavicarpa*, a similar finding was reported using histochemical tests involving alpha-naphthyl acetate and hydrogen peroxide, along with in vivo pollination, to evaluate floral receptivity at six intervals after anthesis (Souza et al. 2004c). A 44% reduction in the percentage of fertilized flowers during in vivo pollination was observed. Conversely, this decline was less pronounced in biochemical tests, showing only

a 20% decrease. An average floral receptivity of 80% was recorded even five hours post-anthesis (Souza et al. 2004c). A similar pattern was observed with pollen viability, which decreased by 15% after anthesis (Souza et al. 2002).

The results regarding floral receptivity and pollen viability after anthesis (Souza et al. 2002, 2004c) help explain the reduction in the number of fertilized flowers during *P. edulis* self-pollination. Similarly, the decline in fertilized flowers after anthesis in *P. cacao* could also be attributed to the reduced viability of *P. edulis* pollen, as its viability typically decreases the day following anthesis (Souza et al. 2002).

As considerations, the cytogenetic studies were conducted on both *P. cacao* and *P. edulis* and the results revealed differences between the species concerning the positioning of satellites, the presence of constitutive heterochromatin (C banding), the locations of 45S rDNA sites, and CMA bands. The cytogenetic evidence suggests that while *P. cacao* shares a close relationship with *P. edulis*, it remains a distinct species (Viana and Souza 2012). *Passiflora edulis* and *P. cacao* are considered genetically close based on pollen characteristics and reproductive abilities. However, distinctions arise from differences in pollen grain ornamentation, such as the presence of pila on the muri and a single row of brochi between two pairs of colpi in *P. cacao*, emphasize the differences between the two species. By examining pollen characteristics, vegetative morphology, and comparing the *trnL* intron sequence, *P. cacao* was placed within the *Passiflora* subgenus group. The *trnL* intron sequence analysis effectively distinguished the two species, confirming the uniqueness of each genotype.

Acknowledgements We thank Dr. Luís Carlos Bernacci for collecting the *Passiflora cacao* germplasm and Dr. Vitor Becker for allowing the collection at the *Reserva Particular do Patrimônio Natural Serra Bonita* (Uiraçu Institute), Camacan, BA, Brazil. We also thanks to the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES), for granting our scholarship, the *Fundação de Amparo à Pesquisa do Estado da Bahia* (FAPEB), the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq), and the *Universidade Estadual de Santa Cruz* (UESC) for the financial support.

Author contributions All authors contributed to the study conception and design. Material preparation and data collection were performed by Américo José Carvalho Viana. Data analysis was performed by Margarete Magalhães Souza, Juliane dos Santos Amorim, Ronan Xavier Corrêa, Ioná Santos Araújo Holanda and Dário Ahnert. The first draft of the manuscript was written by Américo José Carvalho Viana, Margarete Magalhães Souza, Juliane dos Santos Amorim, Ronan Xavier Corrêa, Ioná Santos Araújo Holanda, Dário Ahnert and Léonard Jean Victor Nicolas and all authors commented on previous versions of the manuscript. All authors read and approved of the final manuscript.

Funding The Article Processing Charge (APC) for the publication of this research was funded by the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil* (CAPES) (ROR identifier: 00x0ma614).

Data availability Not applicable.

Declarations

Financial interests The authors declare they have no financial interests.

Conflict of interest The authors declare no competing interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Abreu PP, Souza MM, Santos EA, Pires MV, Pires MM, Almeida A-AF (2009) Passionflower hybrids and their use in the ornamental plant market: perspectives for sustainable development with emphasis on Brazil. *Euphytica* 166:307–315. <https://doi.org/10.1007/s10681-008-9835-x>
- Amela García MT, Galati BG, Anton AM (2002) Microsporogenesis, microgametogenesis and pollen morphology of *Passiflora* spp. (*Passifloraceae*). *Bot J Linn Soc* 139:383–394. <https://doi.org/10.1046/j.1095-8339.2002.00072.x>
- Amorim JS, Souza MM, Viana AJC, Freitas JCO (2011) Self-, cross- and interspecific pollinations in *Passiflora capsularis* and *P. rubra*. *Rev Bras Bot* 34:525–532. <https://doi.org/10.1590/S0100-84042011000400007>
- Amorim JS, Souza MM, Viana AJC, Corrêa RX, Araújo IS, Ahnert D (2014) Cytogenetic, molecular and morphological characterization of *Passiflora capsularis* L. and *Passiflora rubra* L. *Plant Syst Evol* 300:1147–1162. <https://doi.org/10.1007/s00606-013-0952-1>
- Araújo RCMS, Santos FAR (2004) Palinologia de espécies do gênero *Passiflora* L. (*Passifloraceae*) da Chapada Diamantina, Bahia, Brasil. *Sitientibus Ser Cienc Biol* 4:37–42
- Araújo RCMS, Nunes TS, Saba MD, Andrade BR, Nascimento AP, Santos FAR (2019) Palynotaxonomy of Brazilian species of *Passifloraceae* sensu. *Acta Bot Bras* 3:54–65. <https://doi.org/10.1590/0102-33062019abb0229>
- Barth OM, Melhem TS (1988) *Glossário Ilustrado de Palinologia*. Editora da Unicamp, Campinas
- Bernacci LC, Souza MM (2012) *Passiflora cacao* (*Passifloraceae*), a new species from Southern Bahia, Brazil. *Novon: J Bot Nomenclature* 22:1–7. <https://doi.org/10.3417/2010043>
- Bernacci LC, Soares-Scott MD, Junqueira NTV, Passos IRS, Meletti LMM (2008) *Passiflora edulis* Sims: the correct taxonomic way to cite the yellow passion fruit (and of other colors). *Rev Bras Frutic* 30:566–576. <https://doi.org/10.1590/S0100-29452008000200053>
- Bernacci LC, Cervi AC, Giovanni R, Borges RAX, Hering RLO, Serano T, Santos Filho LAF (2013) *Passifloraceae*. In: Martinelli G, Moraes MA (eds) *Livro vermelho da flora do Brasil*. Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Rio de Janeiro, pp 830–834
- Bernacci LC, Cervi AC, Milward-de-Azevedo MA, Nunes TS, Imig DC, Mezzonato AC (2015) *Passifloraceae*. Lista de espécies da

- flora do Brasil. Jardim Botânico do Rio de Janeiro. <http://flora.dobrasil.jbrj.gov.br/jabot/listaBrasil/PrincipalUC/PrincipalUC.do?lingua=em>. Accessed 19 February 2015
- Bourgoin C, Betbeder J, Roux R, Gond V, Oszwald J, Arvor D, Baudry J, Boussard H, Clech S, Mazzei L, Dessard H, Läderach P, Raymondin L, Blanc L (2021) Looking beyond forest cover: an analysis of landscape-scale predictors of forest degradation in the Brazilian Amazon. *Environ Res Lett* 16:114045. <https://doi.org/10.1088/1748-9326/ac31eb>
- Bruckner CH, Casali VWD, Moraes CF, Regazzi AJ, Silva EAM (1995) Self-incompatibility in passion fruit (*Passiflora edulis* Sims). *Acta Hort* 370:45–57. <https://doi.org/10.17660/ActaHortic.1995.370.7>
- Castillo NR, Ambachew D, Melgarejo LM, Blair MW (2020) Morphological and agronomic variability among cultivars, landraces, and Genebank accessions of purple passion fruit, *Passiflora edulis* f. *edulis*. *HortScience* 55:768–777. <https://doi.org/10.21273/HORTSCI14553-19>
- Cauz-Santos LA, Costa ZP, Callot C, Cauet S, Zucchi MI, Berges H, Berg CVD, Vieira MLC (2020) A repertoire of rearrangements and the loss of an inverted repeat region in *Passiflora* chloroplast genomes. *Genome Biol Evol* 12:1841–1857. <https://doi.org/10.1093/gbe/evaa155>
- Cervi AC, Imig DC (2013) A new species of *Passiflora* (*Passifloraceae*) from Mato Grosso do Sul, Brazil. *Phytotaxa* 103:46–50. <https://doi.org/10.11646/phytotaxa.103.1.3>
- Crochemore ML, Molinari HB, Stenzel NMC (2003) Agro-morphological characterization of passion fruit (*Passiflora* spp.) germoplasm. *Rev Bras Frutic* 25:5–10. <https://doi.org/10.1590/S0100-29452003000100004>
- Cruden RW (1977) Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. *Evolution* 31:32–46. <https://doi.org/10.2307/2407542>
- Cruz CD (2006) Programa Genes: análise multivariada e simulação. Editora UFV, Viçosa
- Cruz CD, Regazzi AJ, Carneiro PCS (2012) Modelos biométricos aplicados ao melhoramento genético. Editora UFV, Viçosa
- Dafni A (1992) Pollination ecology: a practical approach. Oxford University Press, New York
- Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. *Focus* 12:13–15
- Efe F, Sevindik E, Sevindik M, Paksoy MY (2025) Phylogenetic analysis of *Lisaea* Boiss. (Apiaceae) species in Türkiye based on chloroplast DNA (cpDNA) *trnL*-intron, *trnL*-F, *matK* and *rbcL* sequences. *Biol Bull* 52:199. <https://doi.org/10.1134/S1062359025600412>
- Erdtman G (1943) An introduction to pollen analysis. Chronica Botanica Company, Waltham
- Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 17:368–376. <https://doi.org/10.1007/BF01734359>
- Gielly L, Taberlet P (1994) The use of chloroplast DNA to resolve plant phylogenies: noncoding versus *rbcL* sequences. *Mol Biol Evol* 11:769–777. <https://doi.org/10.1093/oxfordjournals.molbev.a040157>
- Godoy PRE, Souza MM, Roza FA, Lawinsky PR, Araújo IS, Ahnert D (2009) Pollen performance in self-compatible and self-incompatible cacao trees (*Theobroma cacao* L.). *Rev Bras Bot* 32:617–620. <https://doi.org/10.1590/S0100-84042009003000019>
- Hansen AK, Gilbert LE, Simpson BB, Downie SR, Cervi AC, Jansen RK (2006) Phylogenetic relationships and chromosome number evolution in *Passiflora*. *Syst Bot* 31:138–150. <https://doi.org/10.1600/036364406775971769>
- He X, Luan F, Yang Y, Wang Z, Zhao Z, Fang J, Wang M, Zuo M, Li Y (2020) *Passiflora edulis*: an insight into current research on phytochemistry and pharmacology. *Front Pharmacol* 11:1–16. <https://doi.org/10.5007/2175-7925.2020.e70282>
- Hirao T, Watanabe A, Kurita M, Kondo T, Takata K (2008) Complete nucleotide sequence of the *Cryptomeria japonica* D. Don. chloroplast genome and comparative chloroplast genomics: diversified genomic structure of coniferous species. *BMC Plant Biol* 8:70. <https://doi.org/10.1186/1471-2229-8-70>
- Huang H, Shi C, Liu Y, Mao S-Y, Gao L-Z (2014) Thirteen *Camellia* chloroplast genome sequences determined by high-throughput sequencing: genome structure and phylogenetic relationships. *BMC Evol Biol* 14:151. <https://doi.org/10.1186/1471-2148-14-151>
- Jesus ON, Oliveira EJ, Faleiro FG, Soares TL, Girardi EA (2017) Illustrated morpho-agronomic descriptors for *Passiflora* spp. Embrapa Mandioca e Fruticultura, Cruz das Almas
- Juchum FS, Costa MA, Amorim AM, Corrêa RX (2008) Phylogenetic relationships among morphotypes of *Caesalpinia echinata* Lam. (Caesalpinioideae: Leguminosae) evidenced by *trnL* intron sequences. *Naturwissenschaften* 95:1085–1091. <https://doi.org/10.1007/s00114-008-0424-6>
- Jukes TH, Cantor CR (1969) Evolution of protein molecules. In: Munro HN (ed) Mammalian Protein Metabolism. Academic Press, New York, pp 21–132
- Killip EP (1938) The American species of *Passifloraceae*. Field Museum of Natural History, Chicago
- Krosnick SE, Porter-Utley KE, MacDougal JM, Jørgensen PM, McDade LA (2013) New insights into the evolution of *Passiflora* subgenus *Decaloba* (*Passifloraceae*): phylogenetic relationships and morphological synapomorphies. *Syst Bot* 38:692–713. <https://doi.org/10.1600/036364413X670359>
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 35:1547–1549. <https://doi.org/10.1093/molbev/msy096>
- Li H, Wu M, Lai Q, Zhou W, Song C (2023) Complete chloroplast of four *Sanicula* taxa (Apiaceae) endemic to China: lights into genome structure, comparative analysis, and phylogenetic relationships. *BMC Plant Biol* 23:444. <https://doi.org/10.1186/s12870-023-04447-w>
- Liviston BA, Caetano CM, Cardoso CI, d’Eeckenbrugge GC, Arroyave JA, Olaya CA (2005) Caracterización del polen de especies de los géneros *Passiflora* y *Dilkea*. *Acta Agron* 54:19–23
- Longo D, Lorenz-Lemke AP, Mäder G, Bonatto SL, Freitas LB (2014) Phylogeography of the *Petunia integrifolia* complex in southern Brazil. *Bot J Linn Soc* 174:199–213. <https://doi.org/10.1111/boj.12115>
- MacDougal JM, Feuillet C (2004) Systematics. In: Ulmer T, MacDougal JM (eds) *Passiflora: Passionflowers of the World*. Timber Press, Portland, pp 27–31
- Madureira HC, Pereira TNS, Cunha M, Klein DE, Oliveira MVV, Mattos L, Souza Filho GA (2014) Self-incompatibility in passion fruit: cellular responses in incompatible pollinations. *Biol* 69:574–584. <https://doi.org/10.2478/s11756-014-0353-0>
- MAPA (2008) Instruções para execução dos ensaios de distinguibilidade, homogeneidade e estabilidade de cultivares de *Passiflora*. Ministério da Agricultura, Pecuária e Abastecimento. <http://www.agricultura.gov.br> Accessed 15 Mar 2009
- Meletti LMM, Soares-Scott MD, Bernacci LC (2005) Phenotypic characterization in three selections of purple passion fruit (*Passiflora edulis* Sims). *Rev Bras Frutic* 27:268–272. <https://doi.org/10.1590/S0100-29452005000200020>
- Mezzonato-Pires AC, Mendonça CBF, Milward-de-Azevedo MA, Gonçalves-Esteves V (2017) The systematic value of pollen morphology of *Passiflora* subgenus *Astropheia* (*Passifloraceae*). *Phytotaxa* 298:1. <https://doi.org/10.11646/phytotaxa.298.1.1>

- Mezzonato-Pires AC, Milward-de-Azevedo MA, Mendonça CBF (2020) A taxonomic revision of *Passiflora* subgenus *Astrophea* (*Passifloraceae sensu stricto*) in Brazil. *Phytotaxa* 473:1–60. <https://doi.org/10.11646/phytotaxa.473.1.1>
- Milward-de-Azevedo MA (2019) New records of *Passiflora* subgenus *Decaloba* (*Passifloraceae*). *Check List* 15:149–159. <https://doi.org/10.15560/15.1.149>
- Milward-de-Azevedo MA, Baumgratz JFA (2004) *Passiflora* L. subg. *Decaloba* (DC.) RCHB. (*Passifloraceae*) in Southeastern Brazil. *Rodriguésia* 55:17–54. <https://doi.org/10.1590/2175-78602004558502>
- Milward-de-Azevedo MA, Souza FC, Baumgratz JFA, Gonçalves-Esteves V (2010) Palynotaxonomy of *Passiflora* L. subg. *Decaloba* (DC.) Rchb. (*Passifloraceae*) in Brazil. *Acta Bot Bras* 24:133–145. <https://doi.org/10.1590/S0102-33062010000100013>
- Milward-De-Azevedo MA, Souza FC, Gonçalves-Esteves V, Kinoshita LS (2014) Palynotaxonomy of *Passiflora* section *Xerogona* (*Passifloraceae*). *Phytotaxa* 159:1–10. <https://doi.org/10.11646/phytotaxa.159.1.1>
- Morton BR (2002) The role of context-dependent mutations in generating compositional and codon usage bias in grass chloroplast DNA. *J Mol Evol* 56:616–629. <https://doi.org/10.1007/s00239-002-2430-1>
- Muschner VC, Lorenz AP, Cervi AC, Bonatto SL, Souza-Chies TT, Salzano FM, Freitas LB (2003) A first molecular phylogenetic analysis of *Passiflora* (*Passifloraceae*). *Am J Bot* 90:1229–1238. <https://doi.org/10.3732/ajb.90.8.1229>
- Muschner VC, Lorenz-Lemke AP, Vecchia M, Bonatto SL, Salzano FM, Freitas LB (2006) Differential organellar inheritance in *Passiflora*'s (*Passifloraceae*) subgenera. *Genetica* 128:449–453. <https://doi.org/10.1007/s10709-006-7726-4>
- Nóbrega DS, Peixoto JR, Vilela MS, Faleiro FG, Gomes KPS, Sousa RMD, Nogueira I (2017) Agronomic descriptors and ornamental potential of passion fruit species. *Ornam Hort* 23:357–362. <https://doi.org/10.14295/oh.v23i3.1053>
- Okamoto S, Odashima M, Fujimoto R, Sato Y, Kitashiba H, Nishio T (2007) Self-compatibility in *Brassica napus* is caused by independent mutations in *S*-locus genes. *Plant J* 50:391–400. <https://doi.org/10.1111/j.1365-3113X.2007.03058.x>
- Pádua JG, Oliveira EJ, Zucchi MI, Oliveira GCX, Camargo LEA, Vieira MLC (2005) Isolation and characterization of microsatellite markers from the sweet passion fruit (*Passiflora alata* Curtis: *Passifloraceae*). *Mol Ecol Notes* 5:863–865. <https://doi.org/10.1111/j.1471-8286.2005.01090.x>
- Pereira PPA, Lima LKS, Soares TL, Laranjeira FF, Jesus ON, Girardi EA (2019) Initial vegetative growth and survival analysis for the assessment of *Fusarium* wilt resistance in *Passiflora* spp. *Crop Prot* 121:195–203. <https://doi.org/10.1016/j.cropro.2019.03.018>
- Punt W, Hoen PP, Blackmore S, Nilsson S, Le Thomas A (2007) Glossary of pollen and spore terminology. *Rev Palaeobot Palynol* 143:1–81. <https://doi.org/10.1016/j.revpalbo.2006.06.008>
- R Development Core Team (2008) A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Ramaiya SD, Bujang JS, Zakaria MH, Saupi N (2019) Nutritional, mineral and organic acid composition of passion fruit (*Passiflora* species). *Food Res* 3:231–240. [https://doi.org/10.26656/fr.2017.3\(3\).233](https://doi.org/10.26656/fr.2017.3(3).233)
- Rêgo MM, Bruckner CH, Silva EAM, Finger FL, Siqueira DL, Fernandes AA (1999) Self-incompatibility in passion fruit: evidence of two locus genetic control. *Theor Appl Genet* 98:564–568. <https://doi.org/10.1007/s001220051105>
- Rêgo MM, Rêgo ER, Bruckner CH, Finger FL, Otoni WC (2013) Overcoming self-incompatibility in passion fruit by double pollination in anthesis stages. *Acta Hort* 1000:533–536. <https://doi.org/10.17660/ActaHortic.2013.1000.76>
- Rezende CL, Scarano FR, Assad ED, Joly CA, Metzger JP, Strassburg BBN, Tabarelli MGA, Fonseca GA, Mittermeier RA (2018) From hotspot to hopespot: an opportunity for the Brazilian Atlantic Forest. *Perspect Ecol Conserv* 16:208–214. <https://doi.org/10.1016/j.pecon.2018.10.002>
- Santos EA, Souza MM, Abreu PP, Conceição LDHCS, Araújo IS, Viana AP, Almeida A-AF, Freitas JCO (2012) Confirmation and characterization of interspecific hybrids of *Passiflora* L. (*Passifloraceae*) for ornamental use. *Euphytica* 184:389–399. <https://doi.org/10.1007/s10681-011-0607-7>
- Santos A, Penha H, Bellec A, Munhoz CF, Pedrosa-Harand A, Bergès H, Vieira MLC (2014) Begin at the beginning: a BAC-end view of the passion fruit (*Passiflora*) genome. *BMC Genomics* 15:816. <https://doi.org/10.1186/1471-2164-15-816>
- Santos VO, Viana AP, Preisigke SC, Santos EA (2019) Backcrosses in a segregating population of *Passiflora* mediated by morphoagronomic and resistance traits. *Bragantia* 78:542–552. <https://doi.org/10.1590/1678-4499.20180470>
- Sevindik E, Can AE, Özkara Z, Dugun Y, Toksöz S, Özdemir HI (2024) Phylogenetic relationships among *Lamiaceae* species from Aydın (Türkiye), based on *rbcL* sequences. *Biol Nyssana* 15:2. <https://doi.org/10.5281/zenodo.13902177>
- Singh D (1981) The relative importance of characters affecting genetic divergence. *Indian J Genet Plant Breed* 41:237–245
- Souza MM, Pereira TNS, Martins ER (2002) Microsporogenesis and microgametogenesis with relation to flower bud and anther size, and pollen viability in yellow passion fruit (*Passiflora edulis* Sims f. *flavicarpa* Degener). *Cienc Agrotec* 26:1209–1217
- Souza DAS, Lenzi M, Orth AI (2004a) A contribution to the pollination ecology of *Tabebuia pulcherrima* (*Bignoniaceae*) in a sand-bank area of the south of Santa Catarina State, Brazil. *Biotemas* 17:47–66
- Souza MM, Palomino G, Pereira TNS, Pereira MG, Viana AP (2004b) Flow cytometric analysis of genome size variation in some *Passiflora* species. *Hereditas* 141:31–38. <https://doi.org/10.1111/j.1601-5223.2004.01739.x>
- Souza MM, Pereira TNS, Viana AP, Pereira MG, Amaral Júnior AT, Madureira C (2004c) Flower receptivity and fruit characteristics associated to time of pollination in the yellow passion fruit *Passiflora edulis* Sims f. *flavicarpa* Degener (*Passifloraceae*). *Sci Hortic* 101:373–385. <https://doi.org/10.1016/j.scienta.2003.11.020>
- Souza MM, Viana AP, Pereira TNS (2010) A putative mutant of a self-compatible yellow passion fruit with the corona color as a phenotypic marker. *Bragantia* 69:9–16. <https://doi.org/10.1590/S0006-87052010000100003>
- Souza VO, Souza MM, Silva GS, Melo CAF, Freitas JCO, Souza AC (2020) Morphological and cytogenetic characterization of new ornamental *Passiflora* hybrids (*P. 'Vivis'* and *P. 'Jhovi'*). *Crop Breed Appl Biotechnol* 20:e276920313. <https://doi.org/10.1590/1984-70332020v20n3a47>
- Souza AO, Lewis GP, Da Silva MJ (2021) A new infrageneric classification of the pantropical genus *Chamaecrista* (*Fabaceae*: *Caesalpinioideae*) based on a comprehensive molecular phylogenetic analysis and morphology. *Bot J Linn Soc* 197:350–395. <https://doi.org/10.1093/botlinnean/boab029>
- Spirlet ML (1965) Utilisation taxonomique des grains de pollen de Passifloracées. I. Pollen Spores 7:249–301
- Suassuna TMF, Bruckner CH, Carvalho CR, Borém A (2003) Self-incompatibility in passion fruit: evidence of gametophytic-sporophytic control. *Theor Appl Genet* 106:298–302. <https://doi.org/10.1007/s00122-002-1103-1>
- Taberlet P, Gelly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA.

- Plant Mol Biol 17:1105–1109. <https://doi.org/10.1007/BF00037152>
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739. <https://doi.org/10.1093/molbev/msr121>
- Tang C, Chen X, Deng Y, Geng L, Ma J, Wei X (2022) Complete chloroplast genomes of *Sorbus sensu stricto* (*Rosaceae*): comparative analyses and phylogenetic relationships. BMC Plant Biol 22:495. <https://doi.org/10.1186/s12870-022-03858-5>
- Thanikaimoni G (1986) Pollen apertures: form and function. In: Blackmore S, Fergunson IK (eds) Pollen and spores: form and function. Academic Press, London, pp 119–136
- Tynkevicha YO, Boychuka SV, Shelyfista AY, Chorneya II, Volkov RA (2023) Molecular phylogeny and genetic diversity of Carpathian members of the genus *Muscari* inferred from plastid DNA sequences. Cytol Genet 57:3–16. <https://doi.org/10.3103/S0095452723050079>
- Ulmer T, MacDougal JM (2004) *Passiflora* - passionflowers of the world. Timber Press, Portland
- Upadhyaya HD, Kashiwagi J, Varshney RK, Gaur PM, Saxena KB, Krishnamurthy L, Gowda CLL, Pundir RPS, Chaturvedi SK, Basu PS, Singh IP (2012) Phenotyping chickpeas and pigeon peas for adaptation to drought. Front Physiol 3:179. <https://doi.org/10.3389/fphys.2012.00179>
- Vanderplank J (2000) Passion flowers. The MIT Press, Cambridge
- Viana AJC, Souza MM (2012) Comparative cytogenetics between the species *Passiflora edulis* and *Passiflora cacaoensis*. Plant Biol 14:820–827. <https://doi.org/10.1111/j.1438-8677.2011.00557.x>
- Viana AJC, Souza MM, Araújo IS, Corrêa RX, Ahnert D (2010) Genetic diversity in *Passiflora* species determined by morphological and molecular characteristics. Biol Plant 54:535–538. <https://doi.org/10.1007/s10535-010-0094-z>
- Wu C, Chaw S (2015) Evolutionary stasis in cycad plastomes and the first case of plastome GC-biased gene conversion. Genome Biol Evol 7:2000–2009. <https://doi.org/10.1093/gbe/evv125>
- Wu C, Wang Y, Liu S, Chaw S (2007) Chloroplast genome (cpDNA) of *Cycas taitungensis* and 56 cp protein-coding genes of *Gnetum parvifolium*: insights into cpDNA evolution and phylogeny of extant seed plants. Mol Biol Evol 24:1366–1379. <https://doi.org/10.1093/molbev/msm059>
- Yanasan N, Wangkananon W, Natakankitkul S, Kiattisin K (2024) Nanoemulsions containing *Passiflora quadrangularis* L. fruit extracts for cosmetic application and skin efficacy study. Cosmetics 11:57. <https://doi.org/10.3390/cosmetics11020057>
- Yotoko KSC, Dornelas MC, Togni PD, Fonseca TC, Salzano FM, Bonatto SL, Freitas LB (2011) Does variation in genome sizes reflect adaptive or neutral processes? New clues from *Passiflora*. PLoS ONE 6:e18212. <https://doi.org/10.1371/journal.pone.0018212>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.