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Phylogenetic relationships among Mexican species of the genus *Sechium* (Cucurbitaceae)

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Abstract: The genus *Sechium* includes 11 species, which are highly related to the genus *Sicyos*. *Sechium* is native to Mesoamerica, but only *S. edule* and *S. tacaco* have been domesticated. *Sechium edule* is the most exploited species of the genus due to its broad phenotypic variability. A total of 68 samples from the species *S. chinantlense, S. compositum, S. hintonii* and *S. edule* were evaluated, as well as their respective varietal complexes (*albus*, *nigrum* and *virens* groups), thereby amplifying the *loci* of mitochondrial DNA (*CoxIII*), chloroplast DNA (*rbcL*) and the nuclear ribosomal ITS1-5.8S-ITS2 region. This was done with the aim of establishing phylogenetic relationships that help to understand evolutionary processes, which, in turn, will contribute to the taxonomy of the Mexican clade of *Sechium* and its exploitation in breeding. Phylogenetic trees obtained from Bayesian inference and the maximum parsimony show that *S. chinantlense*, *S. compositum,* and *S. edule* form a cluster with a high phylogenetic kinship. *Sechium chinantlense and S. compositum* could possibly be populations or varieties derived from *S. edule.*

Key words: Evolution, domestication, taxonomy, *loci*, Mexican clade

1. Introduction

The Cucurbitaceae family originated from Asia (Schaefer et al., 2009). The Plant List[1] records 11 accepted species of the genus *Sechium* P. Browne, all of which are one-seeded fleshy or fibrous fruits. *Sechium panamensis*, *S. tacaco*, *S. talamancensis,* and *S. villosum* were first described within the genus *Frantzia* (Wunderlin, 1976), but later they were annexed to *Sechium* (Lira et al., 1999) although, in www.cucurbit.de[2], the genus *Frantzia* is accepted, and the genus *Sechium* been included in the genus *Sicyos*, having high phylogenetic affinity (Sebastian et al., 2012). Lira (1995) lists four Mexican species with their morphology, phenology, and eco-geography: *S. chinantlense* Lira & F. Chiang, *S. compositum* (Donn. Sm.) C. Jeffrey, *S. edule* (Jacq.) Swartz, and *S. hintonii* (Paul G. Wilson) C. Jeffrey. The rest of the genus is made up of *S. mexicanum* Lira & M. Nee, *S. panamense* (Wunderlin) Lira & F. Chiang, *S. pittieri* (Cogn.) C. Jeffrey, *S. talamancense* (Wunderlin) C. Jeffrey, *S. venosum* (L.D. Gómez) Lira & F. Chiang, *S. villosum* (Wunderlin) C. Jeffrey and *S. tacaco* (Pittier) C. Jeffrey.

The species of the genus *Sechium* are widely distributed in Mesoamerica, five species in Mexico (*S. edule, S. compositum, S. chinantlense, S. hintonii*, and *S. mexicanum*) and six in Central America (*S. panamense, S. talamancensis, S. venosum, S. villosum, S. pittieri* and *S. tacaco*) (Wunderlin, 1976; Lira and Chiang, 1992; Lira and Nee, 1999). *Sechium edule* was first domesticated in southwestern Mexico (Newstrom, 1990, 1991; Cross et al., 2006); together with *S. tacaco* (syn.: *Frantzia tacaco*), they are the most frequently exploited species within the genus due to their phenotypical variability, which is a product of domestication (Cadena-Iñiguez and Arévalo-Galarza, 2011; Monge and Loría, 2017).

The genus *Sechium* was described by P. Browne to accommodate the species *S. edule* (Jeffrey, 1966). The genus *Sechium* has been grouped together with the genera *Microsechium, Parasicyos, Sechiopsis, Sicyos* and *Sicyosperma* in the subtribe *Sicyinae* (Lira et al., 1997). The genera *Sechium* and *Sechiopsis* have floral nectaries located at the base of the receptacle (Lira et al., 1997). The internal transcribed spacer (ITS) studies by Cross et al. (2006) indicated that *S. edule, S. chinantlense* and *S. compositum* are genetically very similar species and that the genus is paraphyletic: the Central American species of *Sechium* are

[1] The Plant List (2010). Version 1.1 [online]. Website http://www.theplantlist.org/ [accessed 05 December 2019].

[2] Schaefer, H. (2020). Cucurbit Website version 1 [online]. Website www.cucurbit.de. [accessed 23 March 2021]

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clustered in another clade far from the Mexican species. Therefore, these authors divide the genus into two separate genera (*Sechium* and *Frantzia*). Phylogenetically, these two genera are also distant, and among them, the *Frantzia* species are restricted to Panama and Costa Rica (Sebastian et al., 2012).

Cross et al. (2006) hypothesized that *S. edule* might have originated from interspecific hybridizations. The chromosome number of *S. edule* has been reported by various authors, and the results differ considerably. Mercado et al. (1993) reported n = 13 for *S. edule* from Oaxaca; De Donato and Cequea (1994) reported n = 14 in six cultivars of *S. edule*. These differences may be due to a process known as dysploidy, where the number of chromosomes changes due to various mutations, but without loss or gain of DNA, which can lead to speciation events from a phylogenetic perspective (De Storme and Mason, 2014).

Lira et al. (1999) suggested that *S. edule* includes two subspecies (*S. edule* subsp. *edule* and *S. edule* subsp. *sylvestre*), where the wild subspecies (*S. edule* subsp. *sylvestre*) gave origin to the cultivated subspecies. *Sechium edule* shares morphological characteristics in flowers and fruits with *S. chinantlense* and *S. compositum*; the staminal structure of *S. compositum* is similar to that of *S. edule*, but the branches of its filaments are thinner and straighter. On the other hand, its fruits do not have the distinctive apical cleft like the fruits of *S. edule*. In addition, its chromosomal number is n = 14. *Sechium chinantlense* (n = 15) has an apical cleft like *S. edule*, but they differ morphologically in the staminal structure (Lira et al., 1999) as well as in some palynological features such as the polar axis, shape of the pollen grain and exine thickness (Lira et al., 1994). *Sechium compositum* has a greater affinity to *S. edule* (Newstrom, 1991). *Sechium hintonii* seems to be the farthest species in the Mexican clade (Cross et al., 2006); compared to the other species of the genus, its inflorescences are pendulous and non-erect, and its fruits are small (2.5–3.5 cm long and 2–2.5 cm wide) and brown in colour (Lira, 1995).

In breeding, it is useful to have knowledge of crop wild relatives, which may confer agronomic characteristics of interest to the cultivated species (Dempewolf et al., 2017). Thus, wild populations of *S. chinantlense* and *S. compositum* related to *S. edule* may have harbouring desirable characteristics that can be used in improving cultivated chayote (Lira et al., 1999). Different types of crosses often constitute one of the classic methods of genetic improvement, the success of which will largely depend on the phylogenetic proximity between species. Wild populations of *S. edule* have been reported to have small dark green fruits with a bitter taste; in contrast, domesticated populations may have larger fruits with colours ranging from light green to yellow and with neutral to sweet flavours. The morphological contrast between wild and domesticated populations allows phylogenetic studies that provide clues about their evolution, and these, in turn, must be complemented with molecular studies of mitochondrial, chloroplast and ribosomal DNA (Patwardhan et al., 2014), which evolve differently. Mitochondrial DNA generally evolves more slowly by a third, compared to chloroplast DNA, which evolves 50% slower compared to nuclear DNA (Wolfe et al., 1987).

The objective of this study was to analyse genomic sequences of mitochondrial and chloroplast DNA, and the nuclear ITS1-5.8S-ITS2 region in 68 samples from four Mexican species of *Sechium*, including the varietal complexes of *S. edule* to establish its phylogenetic relationship and be able to understand its evolution and diversification. This will contribute to the knowledge about the origin and formation of *S. chinantlense* and *S. compositum*, as well as maximize the phylogenetic resources of *Sechium* through the formulation of conservation strategies and breeding.

2. Materials and methods

2.1. Genetic material

19 populations from different localities corresponding to *S. chinantlense*, *S. compositum*, *S. hintonii* and the varietal complexes of *S. edule* described by Cadena-Iñiguez et al. (2008, 2011) were studied. The number of individuals varied in each population (Table 1). From the GenBank, accessions DQ535843 and JN560649 were annexed from the *rbcL* locus corresponding to *S. mexicanum* and *S. hintonii*. H-387-GISeM is a breeding product from *S. edule amarus silvestrys* × *S. edule var. virens levis* 290. In addition, "Perla negra" was obtained from *S. edule var. nigrum minor* × *S. edule var. amarus silvestrys* (Cadena-Iñiguez et al., 2013). The passport data of the samples are shown in Table 2.

2.2. DNA extraction and quality

Young leaf buds from each individual were used to carry out the extraction of total DNA through the CTAB protocol (Doyle and Doyle, 1987). The quality of DNA was determined by electrophoresis in 1.2% agarose gels with the buffer Tris-Acetate-EDTA (TAE 0.5 X) (Tris-Base, acetic acid and 0.5 M EDTA (pH 8.0)). DNA was dyed in ethidium bromide at 1 mg L^{-1} and visualized in a Kodak Electrophoresis Documentation and Analysis (EDAS) 290 (Kodak, Ltd. Rochester, New York, USA). DNA was quantified with the Genesys 10 uv spectrophotometer (Thermo Scientific, Waltham Massachusetts, USA), and the samples were then diluted at a concentration of 10 ng μL^{-1} to carry out the PCR.

2.3. PCR conditions for fragment amplification

Three pairs of primers were employed in the PCR: a single pair for the cytochrome c oxidase subunit III (*CoxIII*) of

Table 1. Genetic materials and accession numbers from the GenBank used in this study.

Species (Number of individuals)	Origin	Accession numbers from GenBank		
		CoxIII	*rbcL*	ITS1-5.8S-ITS2
S. edule var. *albus dulcis* (4)	Zaachila, Oaxaca, Méx.	MN984661	MT000109	MT112256
S. edule var. *albus levis* (big) (3)	Zaachila, Oaxaca, Méx.	MN984662	MT000110	MT112257
S. edule var. *albus levis* (normal) (3)	Zaachila, Oaxaca, Méx.	MN984663	MT000111	MT112258
S. edule var. *albus minor* (3)	Huatusco, Veracruz, Méx.	MN984664	MT000112	MT112259
S. edule var. *albus spinosum* (4)	Huatusco, Veracruz, Méx.	---	---	MT112260
S. edule var. *nigrum conus* (3)	Zimatlán, Oaxaca, Méx.	MN984666	MT000114	MT112262
S. edule var. *nigrum levis* (4)	Huatusco, Veracruz, Méx.	---	MT000115	MT112263
S. edule var. *nigrum maxima* (4)	Huatusco, Veracruz, Méx.	MN984667	---	MT112264
S. edule var. *nigrum minor* (3)	Huatusco, Veracruz, Méx.	MN984668	MT000116	MT112265
S. edule var. *nigrum spinosum* (4)	Zimatlán, Oaxaca, Méx.	MN984669	MT000117	MT112266
S. edule var. *nigrum xalapensis* (3)	Zaachila, Oaxaca, Méx.	MN984670	MT000118	MT112267
S. edule var. Perla negra (3)	Huatusco, Veracruz, Méx.	---	MT000119	MT112268
S. edule H-387-GISeM (3)	Huatusco, Veracruz, Méx.	MN984665	MT000113	MT112261
S. edule var. *virens levis* (3)	Coscomatepec, Veracruz, Méx.	MN984673	MT000122	MT112271
S. edule var. *virens levis* (bitter) (4)	Huatusco, Veracruz, Méx.	MN984676	MT000123	MT112273
S. edule (wild) (5)	Huatusco, Veracruz, Méx.	MN984674	---	MT112272
S. chinantlense Lira & F. Chiang (4)	Huatusco, Veracruz, Méx.	MN984671	MT000120	MT112269
S. compositum (Donn. Sm.) C. Jeffrey (4)	Tapachula, Chiapas, Méx.	MN984672	MT000121	MT112270
S. hintonii (Paul G. Wilson) C. Jeffrey (4)	Huatusco, Veracruz, Méx.	MN984675	JN560649	MT112274

mitochondrial DNA (mtDNA), a single pair of chloroplast DNA (cpDNA) corresponding to ribulose bisphosphate carboxylase large chain (*rbcL*) and a single pair for the nuclear ITS1-5.8S-ITS2 region amplified by primers ITS5-ITS4 (Table 3). PCR reactions were performed in the thermal cycler – Techne TC-512 (Bibby Scientific, Vernon Hills, Illinois, USA). Amplification was performed in a volume of 25 mL: 6 mL of DNA (10 ng mL^{-1}), 2.5 mL of buffer (10X), 10 mL of the dNTP mixture (500 mM), 2 mL of $MgCl_2$ (50 mM), 2 mL of each primer (forward and reverse) (10 pM mL^{-1}), and 0.5 mL of DNA Taq Polymerase (5 U mL^{-1}) (Thermo Fisher Scientific, Wilmington, NC, USA). The PCR conditions were as follows: a) a cycle of initial denaturation at 94 °C for 3 min; b) 30 cycles at 94 °C for 1 min, annealing temperature (Tm°C) as a function of the primers (Table 3) for 1 min, 72 °C for 2 min, and c) a final extension cycle at 72 °C for 10 min.

2.4. Electrophoresis and fragment purification

Each separate gene fragment (*CoxIII*, *rbcL* and the nuclear ITS1-5.8S-ITS2 region) was amplified and separated independently in 1.2% agarose gel, a single band corresponding to each gene was obtained at 120 V for one hour. To dye and visualize the bands, the same procedure used to test DNA quality was followed. The size of the bands was determined with the molecular weight marker GeneRuler 1 Kb DNA Ladder (Wilmington, NC, USA). According to the supplier's instructions, the gel fragments were purified using the Zymoclean Gel DNA Recovery Kit (Zymo Research, USA).

2.5. Quantification and sequencing

After purifying the PCR products, they were quantified using a NanoDrop Lite spectrophotometer (Thermo Scientific, Wilmington, NC, USA), and then sent to Macrogen, Maryland, (www.macrogenusa.com) for Sanger sequencing.

2.6. Alignment and statistical analysis

The GenBank sequences of the genera *Microsechium*, *Sicyos, Parasicyos*, *Sechiopsis*, *Cucumis, Citrullus* and *Cucurbita* were considered as outgroups. The reported GenBank sequences from *Sechium* were also incorporated. The accession numbers of the GenBank sequences of the external groups and of some of *Sechium* appear in the phylogenetic trees (Figures 1, 2, 3). For each *locus*, multiple alignments of sequences were performed using the MUSCLE algorithm of MEGA 7.0 software (Kumar et al., 2016). A consensus sequence was obtained with the

Table 2. Passport data of the samples studied.

Samples	Collection numbers	Collector names	Identifier name	Herbarium
S. edule var. *albus dulcis*	274-05, 275-05 285-05, 286-05	JCI, CHAA, VMCS	JCI	Hortorio Herbarium, Colegio de Posgraduados, Montecillo, Texcoco
S. edule var. *albus levis* (big)	287-05, 291-05 366-06	JCI, CHAA, VMCS	JCI	Hortorio Herbarium, Colegio de Posgraduados, Montecillo, Texcoco.
S. edule var. *albus levis* (normal)	295-05, 355-06 293-05	JCI, CHAA, VMCS	JCI	Hortorio Herbarium, Colegio de Posgraduados, Montecillo, Texcoco.
S. edule var. *albus minor*	261-05, 262-05 294-05	JCI, CHAA, VMCS	JCI	Hortorio Herbarium, Colegio de Posgraduados, Montecillo, Texcoco.
S. edule var. *albus spinosum*	522-09, 283-05 284-05, 522-09	JCI, CHAA, VMCS	JCI	Hortorio Herbarium, Colegio de Posgraduados, Montecillo, Texcoco.
S. edule var. *nigrum conus*	331-06, 348-06 351-06	JCI, CHAA, VMCS	JCI	Hortorio Herbarium, Colegio de Posgraduados, Montecillo, Texcoco,
S. edule var. *nigrum levis*	378-07, 392-07 467-09, 263-05	JCI, CHAA, VMCS	JCI	Hortorio Herbarium, Colegio de Posgraduados, Montecillo, Texcoco.
S. edule var. *nigrum maxima*	319-05, 372-06 375-06, 391-07	JCI, CHAA, VMCS	JCI	Hortorio Herbarium, Colegio de Posgraduados, Montecillo, Texcoco.
S. edule var. *nigrum minor*	396-08, 397-08 327-06	JCI, CHAA, VMCS	JCI	Hortorio Herbarium, Colegio de Posgraduados, Montecillo, Texcoco.
S. edule var. *nigrum spinosum*	354-06, 357-06 358-06, 359-06	JCI, CHAA, VMCS	JCI	Hortorio Herbarium, Colegio de Posgraduados, Montecillo, Texcoco.
S. edule var. *nigrum xalapensis*	370-06, 371-06 379-07	JCI, CHAA, VMCS	JCI	Hortorio Herbarium, Colegio de Posgraduados, Montecillo, Texcoco,
Sechium edule var. "Perla negra"	631-12, 631-13 631-14	JCI, CHAA, VMCS	JCI	Hortorio Herbarium, Colegio de Posgraduados, Montecillo, Texcoco.
Sechium edule H-387-GISeM	387-07, 387-09 543-10	JCI, CHAA, VMCS	JCI	Hortorio Herbarium, Colegio de Posgraduados, Montecillo, Texcoco
Sechium edule var. *virens levis*	550-10, 551-10 552-10	JCI, CHAA, VMCS	JCI	Hortorio Herbarium, Colegio de Posgraduados, Montecillo, Texcoco.
Sechium edule var. *virens levis* (bitter)	546-10, 547-10 548-10, 549-10	JCI, CHAA, VMCS	JCI	Hortorio Herbarium, Colegio de Posgraduados, Montecillo, Texcoco.
Sechium edule wild	273-05, 303-05 360-06, 361-06 273-05	JCI, CHAA, VMCS	JCI	Hortorio Herbarium, Colegio de Posgraduados, Montecillo, Texcoco.
Sechium chinantlense	299-05, 385-07 386-07, 390-07	JCI, CHAA, VMCS	JCI	Hortorio Herbarium, Colegio de Posgraduados, Montecillo, Texcoco.
Sechium compositum	401-08, 405-09 406-09, 544-10	JCI, CHAA, VMCS	JCI	Hortorio Herbarium, Colegio de Posgraduados, Montecillo, Texcoco.
Sechium hintonii	418-09, 418-10 418-08, 418-07	JCI, CHAA, VMCS	JCI	Hortorio Herbarium, Colegio de Posgraduados, Montecillo, Texcoco

JCI: Jorge Cadena-Iñiguez, CHAA: Carlos Hugo Avendaño-Arrazate, VMCS: Víctor Manuel Cisneros Solano.

Table 3. Characteristics of the pairs of primers for each locus.

Locus	Primers	Sequence 5'-3'	Tm (°C)	References
CoxIII	Cox3r	F: CTCCCCACCAATAGATAGAG	52	Duminil et al. (2002)
	Cox3f	R: CCGTAGGAGGTGTGATGT	50	Duminil et al. (2002)
rbcL	rbcL1f	F: ATGTCACCACAAACAGAAAC	48	Olmstead et al. (1992)
	rbcL724r	R: TCGCATGTACCTGCAGTAGC	54	Fay et al. (1997)
ITS1-5.8S-ITS2	ITS4	R: TCCTCCGCTTATTGATATGC	50	White et al. (1990)
	ITS5	F: GGAAGTAAAAGTCGTAACAAGG	51	White et al. (1990)

F: forward, R: reverse.

SeaView software (Gouy et al., 2010) from individuals sampled from each population, wherein the observation that the sequences for the same locus were identical resulted in the same sequences being considered as representatives, and were sent to GenBank for storage (Table 1).

Evolutionary history was inferred using the Maximum Parsimony (MP) (Nei and Kumar, 2000). The bootstrap consensus tree inferred from 1000 replicates was used to represent the evolutionary history of the analysed taxa; the MP tree was obtained using the subtree-pruning-and-regrafting (SPR) algorithm with MEGA 7.0 (Kumar et al., 2016). Mr Bayes software version 3.1.2 (Ronquist and Huelsenbeck, 2003) was used for Bayesian inference (BI) analysis based on the calculation of the posterior probability of a tree. The BI was constructed using the general time-reversible with gamma distribution plus invariant sites (GTR + G + I) since it is one of the most complex models under stationary, reversible, and homogeneous conditions, using a different parameter to model each of the substitutions between nucleotides and their frequencies (Nei and Kumar, 2000). The Markov Chain Monte Carlo (MCMC) algorithm was used, which has four chains at a temperature of 0.5 (one cold chain and three hot chains), the hot chains raise the subsequent probability of the trees to a power between 0 and 1, which favours the jump between the local peaks of this probability; during the search, there is an exchange of chains, and, in this way, the chain with the highest probability behaves as a cold chain and the others as hot ones; this leads to an exhaustive search for a set of possible trees. To determine the divergence of the chains, the average standard deviations and the differences between them were analysed at a value close to or less than 0.01. The analysis was carried out on 100,000 generations with trees sampled every 100 generations (Ronquist and Huelsenbeck, 2003).

3. Results

A total of 179 sequences were obtained for the *CoxIII* (57), *rbcL* (55) and ITS1-5.8S-ITS2 (67) *loci*. However, a consensus sequence of the individuals per population was obtained, thereby determining a high degree of kinship between the sequences, and, thus, it was able to visualize them in the phylogenetic trees together with the outgroups. Except for *coxIII*, the rest of the loci have conserved sequences in more than 50%; even so, *CoxIII* showed a greater number of parsimony-informative sites (32%) (Table 4).

3.1. *CoxIII* phylogenetic analysis

There was no outgroup due to the absence of sequences in the GenBank for *Sechium* species or related genera; therefore, the phylogenetic tree could not be rooted and only the relationship between the accessions was represented regardless of their evolutionary line. The phylogenetic tree with more than 90% support was obtained using the MP method (Figure 1), and the BI tree showed low posterior probability in its branches, which is inconsistent with MP tree. *Sechium hintonii* was far from the rest of the accessions; however, *S. hintonii* accession is related to *S. edule* wild accession. The varietal complexes of the *albus* and *nigrum* groups of *S. edule* are found with *S. compositum* and *S. chinantlense* (Figure 1); the rest of the *nigrum* varietal groups and the wild accession of *S. edule* are clustered. The hybrid H-387-GISeM showed more affinity with the varietal groups of *albus*.

rbcL phylogenetic analysis

The phylogenetic tree was rooted in the genus *Cucurbita*, which belongs to the Cucurbiteae tribe and, in molecular terms, is notably separate from the *Sicyoeae* tribe to which some genera such as *Sechium*, *Sicyos* and *Sechiopsis* belong (Schaefer and Renner, 2011). The genera *Sicyos* and *Sechiopsis* are very close to each other and with *Sechium*, but not so for the genera *Cucurbita* and *Cucumis*, which were separated considerably with low values of booststrapping in the branches (Figure 2) of the MP tree; in the *Sechium* species and varietal complexes, values greater than 90% of support were obtained in the corresponding branches. The phylogenetic tree BI showed polytomies in its branches (tree not shown), which makes it difficult to resolve the evolutionary relationships in the Mexican species of *Sechium*, this statement is also

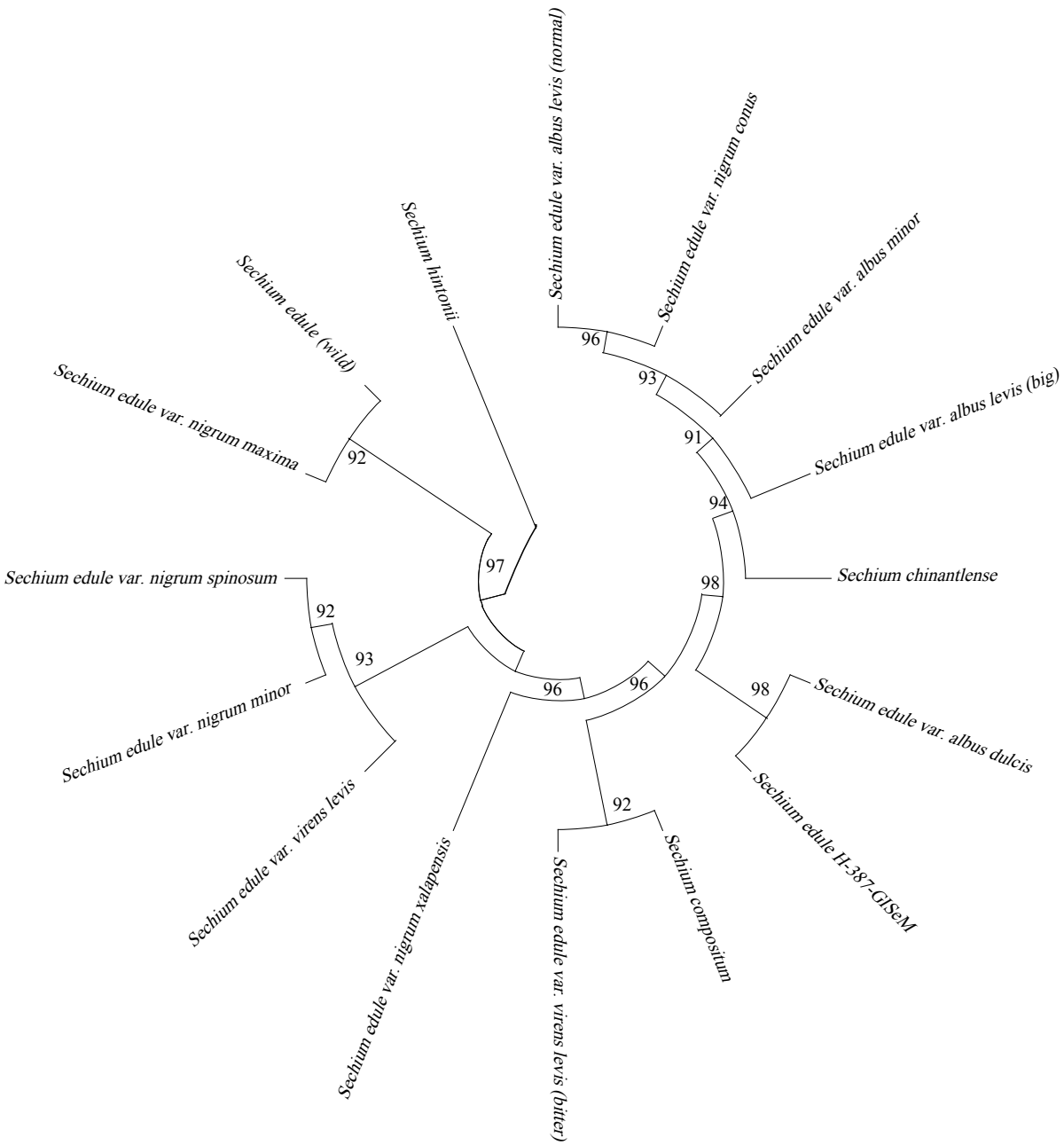

Figure 1. Phylogenetic tree resulting from *coxIII* sequences. The bootstrap consensus tree inferred from 1000 replicates is used to represent the evolutionary history of the analyzed taxa. The percentage of replicate trees, in which the associated taxa are clustered together in the bootstrap test, is shown next to the branches, displaying 50 most parsimonious trees (length = 1502); the consistency index is 0.7 with a retention index of 0.6.

supported by the molecular works of Cross et al. (2006), whose ITS molecular data also record polytomies. The species *S. chinantlense, S. compositum* and some varietal complexes of *albus* group of *S. edule* were closely clustered. The hybrid H-387 GISeM showed a greater affinity with varietal groups of ancestral characteristics, such as *nigrum minor* and *nigrum spinosum*, otherwise, it is clustered with *virens levis*, which is one of its parents.

3.2. ITS region phylogenetic analysis

The phylogenetic tree was rooted in *Parasicyos dieterleae*, in agreement with the phylogram by Sebastian et al. (2012), where this species is rooted in the tree corresponding to the *Sicyoeae* tribe. The BI and MP trees showed support values above 0.5 for the outgroups corresponding to the *Sicyos*, *Microsechium*, *Parasicyos* and *Sechiopsis* species, but not for the *Sechium* species and their varietal complexes, thereby

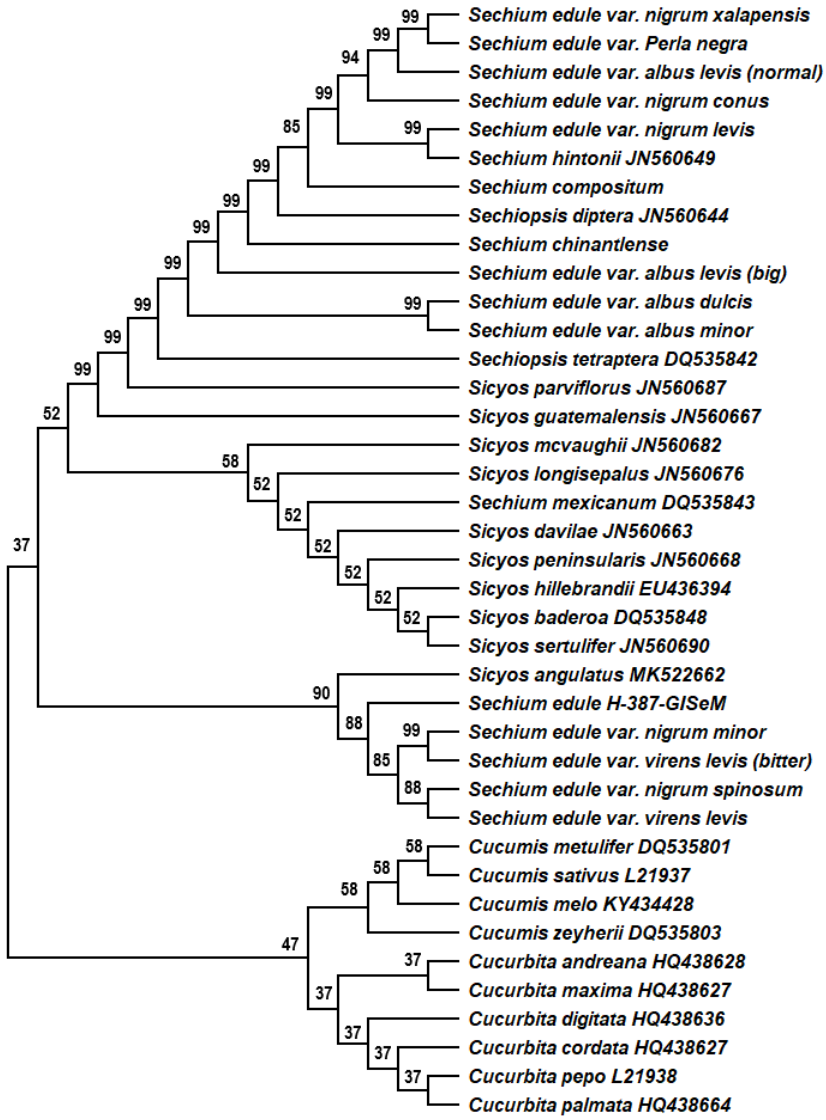

Figure 2. Phylogenetic tree resulting from *rbcL* sequences. The bootstrap consensus tree inferred from 1000 replicates is used to represent the evolutionary history of the analyzed taxa. The percentage of replicate trees, in which the associated taxa are clustered together in the bootstrap test, is shown next to the branches. The parsimony analysis yielded 194 most parsimonious trees with a length of 1542; consistency index being 0.86 with a retention index of 0.77.

obtaining low support values for some nodes (Figure 3). However, the nuclear ITS1-5.8S-ITS2 region remained partially consistent with the *CoxIII* and *rbcL loci*, that is to say, the Mexican species of *Sechium* and the varietal complexes of *S. edule* form a well differentiated group from the external groups. In addition, *S. chinantlense* and *S. compositum* are phylogenetically related to the varietal complexes of *S. edule. Sechium mexicanum* was grouped quite near species of the genus *Sicyos* (*S. microphyllus, S. angulatus, S. weberbaueri* and *S. sertulifer*), maintaining concordance with the molecular studies by Sebastian et al. (2012). The hybrid H-387 GISeM showed a greater affinity with *nigrum maxima* and *S. hintonii* accessions.

4. Discussion

4.1. Phylogenetic relationships of Mexican species of *Sechium*

The *CoxIII* mitochondrial phylogeny (Figure 1) shows that the *Sechium* varietal complexes of the *albus* group are related to *S. chinantlense* and *S. compositum*. The *albus* group corresponds to chayotes with the lowest degree of domestication because they are not very distant genetically from the wild ancestor and is considered to be of recent divergence with evolutionary characteristics, such as the development of pubescence in stems and leaves, enabling them to adapt to extreme conditions of luminosity and temperature (Cadena-Iñiguez et al., 2008). Likewise, the

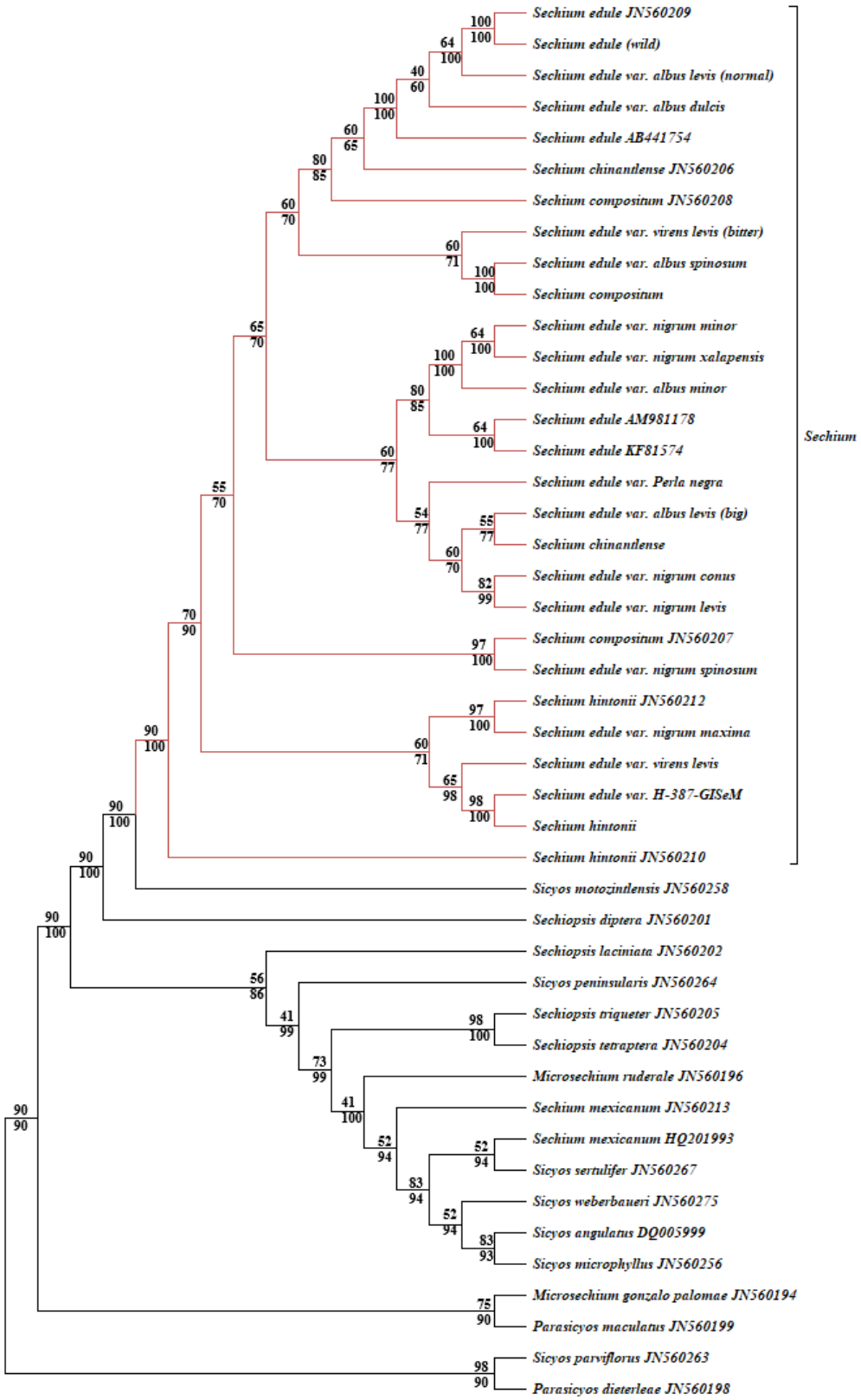

Figure 3. BI phylogenetic tree resulting from ITS1-5.8S-ITS2 sequences obtained from 100,000 generations. The posterior probabilities are indicated below the nodes, and the maximum values of parsimony bootstrap support appear above the nodes. The parsimony analysis yielded 83 most parsimonious trees with a length of 590; consistency index is 0.76 with a retention index of 0.77.

Table 4. General summary of the genetic parameters.

Locus	LAPB	CS (%)	VS (%)	PIS (%)
CoxIII	1223	305 (25)	544 (44)	394 (32)
rbcL	1559	909 (58)	516 (33)	175 (11)
ITS1-5.8S-ITS2	737	378 (51)	320 (43)	172 (23)

LAPB: longitudinally aligned paired base, CS: conserved sites VS: variable sites, PIS: parsimony-informative sites.

hybrid H-387-GISeM is a recent formation, where one of its parents is *S. edule* var. *virens levis* and the other parent is *S. edule amarus silvestrys* (but not included in this study). *Sechium compositum* and *S. chinantlense* are considered different species from *S. edule*. However, phylogenetic studies have indicated that these three species are close to each other and recently evolved within the Mexican clade of *Sechium* (Cross et al., 2006; Sebastian et al., 2012). The molecular study with AFLP by Cross et al. (2006) points out that *S. compositum*, *S. chinantlense* and *S. edule* are different at the species level, but with a certain degree of kinship, that is, the populations of *S. compositum* resemble the populations of *S. edule* from Veracruz and the populations of *S. chinantlense* with the populations of *S. edule* from Chiapas and Guerrero. Nuclear DNA is used to obtain different molecular markers, including AFLPs; however, nuclear DNA evolves faster compared to foreign DNA, which is why their use for studying phylogeny has little recommendation, although they can give some evidence of hybridizations. The first phylogenetic inferences of the *Sechium* species were made on morphological markers, which are susceptible to processes such as domestication and environmental effects. On the other hand, it is possible to have different perspectives on the taxonomy of *Sechium* depending on the geographical region from which the specimens were analysed since there is also the risk that some of them are the product of hybridization. The varietal complexes of the *nigrum* group are closer to wild populations of *S. edule*, and these complexes were considered the closest to the wild relatives in as much as morphological (dark green colour fruits, small and with thorns) and phytochemical characteristics (chlorophyll and cucurbitacin content) (Cadena-Iñiguez et al., 2008, 2011) are concerned. *Sechium hintonii* and wild *S. edule* are phylogenetically close, supporting the results of Cross et al. (2006).

The *rbcL* chloroplast phylogeny (Figure 2) shows that most of the *Sechium* accessions are grouped into a single well-differentiated group of *Sicyos* and *Sechiopsis*. The accessions of *Sechiopsis* and *Sicyos* were grouped in *Sechium*; however, these three genera are included in the *Sicyoeae* tribe, and even Sebastian et al. (2012) argue that all the species form the numerous genus *Sicyos*, thus, excluding the Central American species of *Sechium* (*S. panamense, S. pittieri, S. talamancense, S. tacaco, S. venosum* and *S. villosum*), which had been recorded previously under the genus name *Frantzia*, although presently this genus is not recognized, and it was annexed to the genus *Sechium*. The Central American clade of *Sechium* includes species that are endemic to Panama and Costa Rica, which has been isolated and genetically differentiated from the rest of the species of *Sechium* and the tribe *Sicyoeae* due to geographic issues (Cross et al., 2006; Sebastian et al., 2012). Some varietal complexes of *S. edule* were grouped into pairs or very near to one another with good values of bootstrap support, such as *nigrum xalapensis* and *nigrum conus, nigrum minor, virens levis* (bitter) and *nigrum spinosum*, which have physical and chemical characteristics related to ancestral populations of *S. edule*. The *S. mexicanum* accession clustered with *Sicyos* and *Sechiopsis*. Cross et al. (2006) and Sebastian et al. (2012) also obtained the same results and the latter concluded that *Sechium mexicanum* should be lumped in a broadly circumscribed *Sicyos*. *Sechium hintonii* and *S. mexicanum* are the most ancient within the Mexican clade of *Sechium*, with a molecular clock divergence time estimates for *S. hintonii* to be 5 million years, and 15 million years for *S. mexicanum* (Sebastian et al., 2012).

In the ITS1-5.8S-ITS2 phylogeny (Figure 3), all *Sechium* accessions formed a well-differentiated clade from the genera belonging to the *Sicyoeae* tribe, from which it was inferred through nuclear and plastid sequences, which are monophyletic genera (Schaefer and Renner, 2011; Sebastian et al., 2012). The whole intraspecific variation of *Sechium edule* was grouped correctly with *S. chinantlense* and *S. compositum* according to the ITS phylogeny, which are quite near each other (Cross et al., 2006; Sebastian et al., 2012). The phenotypical diversity of *S. edule* has been product of the interaction of its populations with the environment and with human groups; on the other hand, the possible hybridizations of *S. edule* with *S. chinantlense* and *S. compositum* could also have been participated in the origin of this variation in the populations of *S. edule*. *Sechium hintonii* is only distributed in the Mexican state of Guerrero; its fruits are small compared to *S. edule* and lack an apical cleft (Lira et al., 1999). It also shows differences with Mexican species of *Sechium* with respect to pollen morphology: polar axis, equatorial axis and length of spines. It was originally classified as *Microsechium hintonii*.

4.2. Evolution, diversification and breeding of *Sechium edule*

The ITS phylogeny shows one clade of *Sechium* that is separated from the outer groups *Sicyos*, *Parasicyos*, *Sechiopsis*, and *Microsechium*; the *rbcL* phylogeny shows

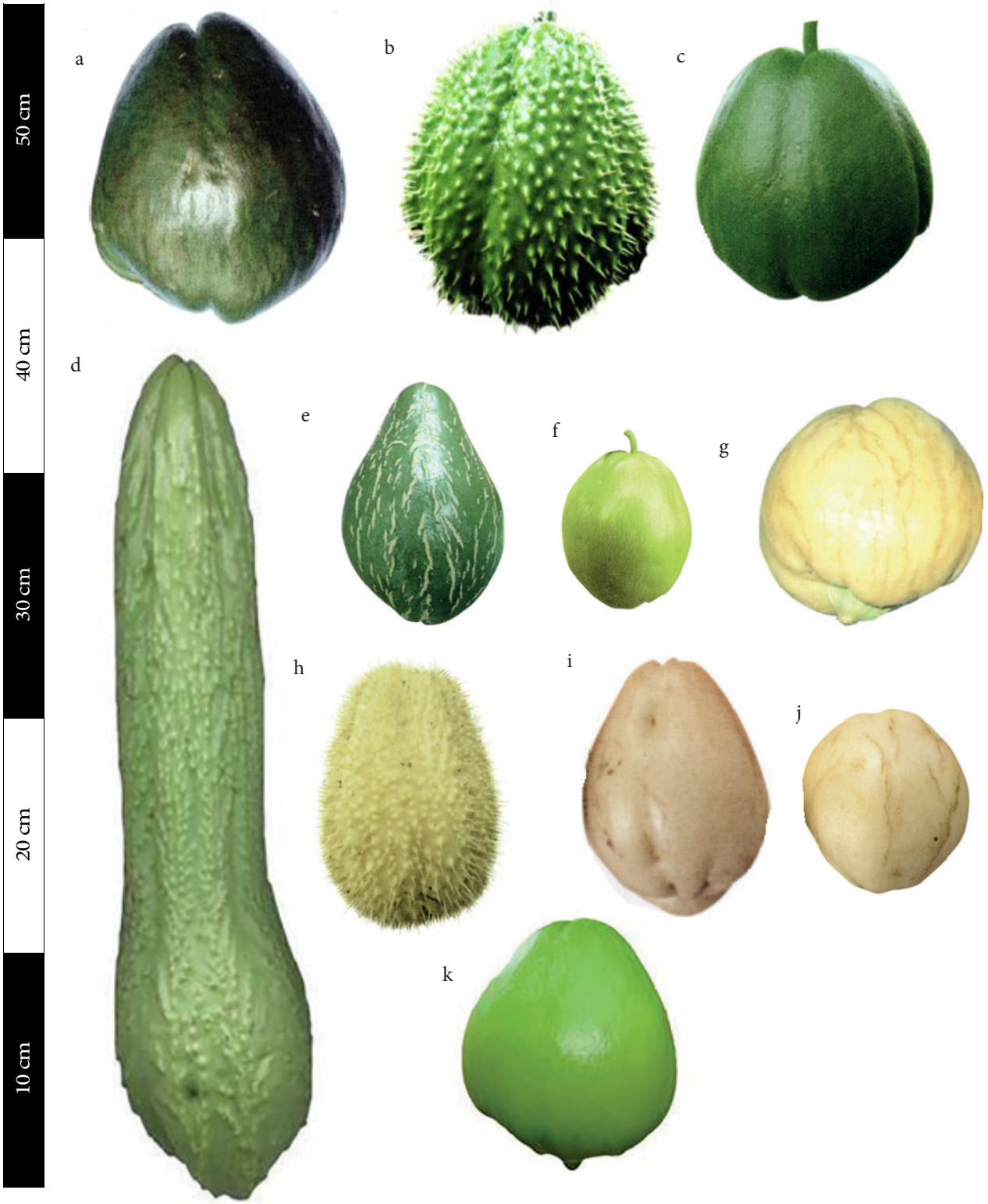

Figure 4. Infraspecific variation of *S. edule*. a. *nigrum xalapensis*. b. *nigrum spinosum*. c. *nigrum levis*. d. *nigrum maxima*. e. *nigrum conus*. f. *nigrum minor*. g. *albus levis*. h. *albus spinosum*. i. *albus dulcis*. **j.** *albus minor*. **k.** *virens levis*. Photographs of Jorge Cadena-Iñiguez.

Figure 5. Fruit types. a. *Sechium edule*. b. *Sechium compositum*. c. *Sechium chinantlense*. d. *Sechium hintonii*. Photographs of Jorge Cadena-Iñiguez.

a differentiated *Sechium* clade but with the inclusion of species of *Sechiopsis* and *Sicyos*. Both phylogenetic trees (*rbcL* and ITS) do not have good support for bootstrap on some branches. The discrepancies between these groupings are due to different mutation rates between genes; as mtDNA generally evolves more slowly by a third, compared to cpDNA, which evolves 50% slower compared to nuclear DNA, that is, a DNA that is highly conserved over time or that has undergone few mutations, can help make phylogenetic inferences of the species and would not be so affected by morphological changes that botanical structures can undergo (Wolfe et al., 1987). However, both mutation rates in nuclear and extra-nuclear DNA can vary as a function of the different lineages of plants (Stenøien, 2008; Yao et al., 2019).

Accessions of wild *S. edule* studied with ITS show certain similarity to *S. hintonii*, possibly because the accessions of the said species were collected in Guerrero, Mexico, and perhaps spontaneous crosses occurred, which explain their similarity (Cross et al., 2006). These four species form a well-differentiated clade in comparison with all species of the Central American clade of *Sechium*.

The earliest populations of *S. chinantlense* and *S. compositum* might have been different; despite the difficulty that exists to demonstrate the natural hybridization of these two species with S. *edule*, Cadena-Iñiguez et al. (2013) have made artificial hybridizations, obtaining new phenotypic variants.

The chromosome numbers of *S. compositum, S. chinantlense* and *S. edule* vary considerably; n = 14 for *S. compositum*, n = 15 for *S. chinantlense* (Lira, 1999), and there are reports of n = 13 and 14 for *S. edule*, which depends on the geographical zone of the samples collected (Mercado et al., 1993; De Donato and Cequea, 1994). Although these conditions may be a barrier to genetic drift and breeding between these species, in dysploidy, DNA reordering due to irregularities in the meiotic division can alter the number of chromosomes, thereby conserving the same genetic load (De Storme and Mason, 2014). This will, in some way, ensure the success of hybridization resulting

in stable and improved genotype results (Cadena-Iñiguez et al., 2013).

Varietal complexes of *S. edule* have diversified over time through the effects of adaptive specialization, as well as natural and artificial selection processes (Figure 4). The varietal complexes of the *nigrum* group are the oldest and closest to a wild ancestor; however, due to the multiple phenotypic variants that exist in the state of Veracruz, Mexico, it is necessary to evaluate even more populations and individuals that help to understand the evolutionary history of the wild populations of *S. edule*. Meanwhile, the varietal complexes of the *albus* group are the most recently divergent. The species *S. chinantlense*, *S. compositum, S. hintonii* and *S. edule* formed a cluster within the Mexican clade of *Sechium* although their fruits are similar; only in *S. edule* there are variants with prickly fruits (Figure 5). Populations of *S. edule* and its varietal complexes could have originated or participated in the formation of *S. chinantlense* and *S. compositum,* and these in turn could be included as *S. edule* variants. The phylogenetic affinity detected between these three species (*S. chinantlense, S. compositum* and *S. edule*) suggests the possibility of carrying out breeding between them.

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