REVIEW ARTICLE

Phylogenetics of the genus *Sechium* P. Brown: A review

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Abstract

The *Sechium* P. Br. genus composed of 11 species, which originated from the mountainous regions of Mesoamerica, have been domesticated and diversified. These species are clustered in two large groups: the Mexican clade and the Central American clade. Morphological and molecular studies have shown that species of the Mexican clade are formed through interspecific hybridizations and genetic flow, with the exception of *S. mexicanum*, which is strongly linked to the genus *Sicyos*. The objective of this review was to analyze the phylogenetics of Sechium based on morphological and molecular studies, which contributed to taxonomic knowledge and utilization, thereby favoring its conservation and improvement. The Central American clade is well supported with molecular data, but not so with morphological data. The species in this clade were geographically isolated and endemic. *S. edule* and *S. tacaco* are exploited species in the agricultural and industrial sectors, and both have an extensive genetic and phenotypic diversity that has allowed them to diversify and expand into different ecological niches. Finally, the Central American species of *Sechium* thrive in adverse environments of temperatures of mesophyll forest and high relative humidity, with characteristics that can give resistance to frosts and phytopathogenic agents, as well as cultivated species of this genus.

Additional key words: genetic flow; hybridizations; genetic diversity; ecological niches; conservation; breeding

Abbreviations used: AFLP (Amplified Fragment Length Polymorphism); ETS (External Transcribed Spacer); ITS (Internal Transcribed Spacer); PCA (Principal Component Analysis).

Introduction

Jeffrey (1966) reported for the first time that the botanical Cucurbitaceae family consists of about 700 species that were distributed in 90 genera. Currently, there are around 1000 accepted species included in 134 genera (The Plant List, 2010), among which the following genera are prominent due to their economic importance: *Cucurbita, Cucumis, Citrullus, Luffa, Lagenaria, Momordica, Trichosanthes, Benincasa,* and *Coccinia*; in these, 10 cultivated and domesticated species are of economic importance since they are cultivated in different parts of the world and are considered as main crops (*Cucurbita argyrosperma, Cucurbita maxima, Cucurbita moshata, Cucurbita pepo, Citrullus lanatus, Benincasa hispida, Cucumis melo, Cucumis sativus, Lagenaria siceraria*, and *Momordica siceraria*); 23 more are listed as crops of local importance and are cultivated, but some of them are not fully domesticated, including *Sechium edule*, which is particularly cultivated in various parts of the world as an introduced species (Chomicki *et al.*, 2019). The Cucurbitaceae family is estimated to have originated on the Asian continent approximately 60 million years ago and later spread to other continents (Schaefer *et al.*, 2009).

The genus *Sechium P.* Brown genus includes the following species: *S. chinantlense* Lira & F. Chiang, *S. compositum* (Donn. Sm) C. Jeffrey, *S. edule* (Jacq.) Swartz, *S. hintonii* (P.G. Wilson) C. Jeffrey, *S. mexicanum* Lira & Nee, *S. panamensis* (Wunderlin) Lira & F. Chiang, *S. pittieri* (Cogn.) C. Jeffrey, *S. tacaco* (Pittier) C. Jeffrey, *S. talamancensis* (Wunderlin) C. Jeffrey, *S. venosum* (L.D. Gómez) Lira & Chiang and *S. villosum* (Wunderlin) C. Jeffrey. These species originated, were domesticated and diversified in Mesoamerica (Newstrom, 1986, 1990, 1991; Lira & Chiang, 1992; Lira, 1995; Lira *et al.*, 1997a; Lira & Nee, 1999; Cross *et al.*, 2006).

The importance of the genus is based on the utilization of its morphological structures (fruits, roots, leaves) for dietary and medicinal purposes (Newstrom, 1990; Lira, 1995, 1996). Cucurbitacins, one of its chemical components, are tetracyclic terpenes present in Cucurbitaceae, and they confer a bitter flavor on its morphological structures and show antitumor activity against several types of cancer (Zhou *et al.*, 2019). The fruits of *S. edule* have been studied with greater accuracy due to their alcoholic compounds possessing anti-neoplastic properties (Monroy-Vázquez *et al.*, 2009; Cadena-Iñiguez *et al.*, 2013b; Aguiñiga-Sánchez *et al.*, 2015, 2017; Salazar-Aguilar *et al.*, 2017).

The *S. edule* and *S. tacaco* species are the most exploited of their genus in the agricultural sector. In particular, the populations of the first species show great phenotypic and genotypic variability, which is the product of the effects of domestication and interaction with the environment, and have been grouped into varietal complexes through secondary morpho-biochemical, stable and heritable characteristics, thereby differentiating each group. Some of the characteristics are the color, shape, texture, flavor, bromatological content of the fruits (Cadena-Iñiguez *et al.*, 2008, 2011; Cadena-Iñiguez & Arévalo-Galarza, 2011), genetic diversity (Machida-Hirano *et al.*, 2015) and cytogenetic structure (Palacios, 1987; Mercado *et al.*, 1993; De Donato & Cequea, 1994; Olvera-Vázquez *et al.*, 2019).

The remaining nine species of the genus *Sechium* have been briefly described in terms of morphology and ecogeography. Some Central American species (S. *talamancensis, S. panamensis, S. pittieri, S. venosum, S. villosum* and *S. tacaco*) have agronomic characteristics that can be transferred to cultivated chayote (Lira, 1995). The recognition of wild ancestors or crop-related species is of vital importance in undertaking genetic improvement programs. However, morphological divergences between wild and cultivated species can cause taxonomic problems due to the existence of plesiomorphic and apomorphic states. The ribosomal and plastid DNA sequences have low mutation rates and are free of selection (Patwardhan *et al.*, 2014), and their study has made people aware of the events of speciation, domestication, diversification and hybridization, which have helped to improve the taxonomy of species (Sanjur *et al.*, 2002; Smýkal *et al.*, 2015; Chen *et al.*, 2016; Zhang *et al.*, 2017).

Understanding the phylogenetic characteristics of species is important for crop improvement. In general, wild species have extensive genetic variability, enabling them to adapt and survive in the face of adverse environmental impacts (Doebley *et al.*, 1987; EL-Bakatoushi & Ahmed, 2018; Gao *et al.*, 2018). Genes of interest can be transmitted to cultured populations using different improvement methods or genetic engineering techniques; however, the success of the incorporation of new genes depends on the phylogenetic proximity between species, thereby ensuring that the progeny are fertile and stable over time (Patwardhan *et al.*, 2014).

Origin and ecogeography

By analyzing the chloroplast genes ribulose biphosphate carboxylase large chain (*rbcL*), maturase-K (*matK*), *trnL* and *trnL-F* and *rpl20-rps*12 intergenic spacers in 240 species from 114 genera of the Cucurbitaceae family in conjunction with a parsimony-based biogeographic analysis to determine vicarious and dispersal events, Asia was found to be the most likely center of origin for lineages in cucurbits and was gradually distributed to the rest of the continents through transoceanic dispersions (Schaefer *et al.*, 2009).

Linguistic evidence, the endemism of most *Sechium* species, the morphological diversity they present, and the existence of wild populations suggest that all 11 *Sechium* species originated and were diversified and domesticated in Mesoamerica (Newstrom, 1990, 1991; Lira & Chiang, 1992; Lira, 1995; Lira *et al.*, 1997b; Lira & Nee, 1999; Cross *et al.*, 2006; Cadena-Iñiguez *et al.*, 2008, 2011, Avendaño-Arrazate *et al.*, 2012, 2017). In Mexico and Guatemala, the *S. edule, S. mexicanum, S. compositum, S. chinantlense* and *S. hintonii* species are most prominent; in Central America, the following species are found: *S. panamensis, S. talamancensis, S. venosum, S. villosum, S. pittieri* and *S. tacaco*. Most Central American species have high degrees of endemism, in which the altitudinal factor influences the characteristics of the ecological niche where each of them develops (Wunderlin, 1976; Lira, 1995; Lira *et al.*, 1999; Lira & Nee, 1999). The fruits, roots and leaves of *Sechium* are widely used in gastronomy (Newstrom, 1990; Lira, 1995) due to the contribution of water, vitamins, sugars, minerals, fiber and carbohydrates (Aung *et al.*, 1990; Lira, 1996; Islam *et al.*, 2018); otherwise, fruits are also used in the industry for the elaboration of flour used in pig meal (Lalthansanga & Samanta, 2015) and as a source of endothelin, which is a powerful vasoconstrictor and mitogen that can be used as a skin-whitening agent (Okamoto *et al.*, 2008).

S. edule. It is the most representative species of the *Sechium* genus due to its agricultural importance, and is known as "chayote", which is a word originally from the Nahuatl term *huizt ayotl* (spiny squash), later *chayotl* and presently chayote. The broad morphological and genetic wealth of *S. edule* in southeastern Mexico and Guatemala, in addition to the presence of S. compositum, which is a highly related wild species, indicates that chayote was domesticated in the region of Chiapas-Oaxaca, Mexico (Newstrom, 1991).

Cadena-Iñiguez *et al.* (2011) studied eight populations of S. edule (seven cultivated and one wild) from

Veracruz, Mexico. Biochemical studies have shown that the wild accession synthesizes a greater quantity of chlorophyll and cucurbitacins with respect to the cultivated populations; principal component analysis (PCA) separated chayote populations by color, texture, and secondary metabolite content. Using the same populations as *S. edule*, Cadena-Iñiguez *et al.* (2008) evaluated them via 27 morphological variables of leaves, flowers and fruits. The PCA for the first component was tilted for the variables of leaf shapes, trichomes, fruit color, flower size and color, petiole color, and vein structure; while for component two, a greater variation was obtained for the characteristics of the fruit (length, width and height). A cladistic analysis based on morphology and anatomy showed that the wild population was the most remote but most likely ancestor of cultivated chayotes. The bitter flavor of the fruits is a characteristic of the wild populations of cucurbits, in which Yuan *et al.* (2016) compared the cucumber, melon and watermelon genomes, thereby finding a group of genes that regulate the synthesis of cucurbitacins and that due to domestication and selection; therefore, wild populations can significantly reduce the bitterness of their fruits.

Castro *et al.* (2015) evaluated the morphological and biochemical diversity of seven cultured *S. edule* accessions in Spain. The length of the fruit had an interval of 9.2-13.1 cm, as Cadena *et al.* (2008) reported an upper limit interval of 3-15 cm, in which other variables such as fruit weight and seed morphometry were also affected by fruit length. However, Castro *et al.* (2015) found a great diversity in the color of the fruit, from light green to dark green; on the other hand, Castro *et al.* (2015) obtained an average of 2.27 ºBrix, which was relatively low compared to that of Cadena *et al.* (2011), who obtained values higher than 10 ºBrix, which is due to the presence of chayotes with yellow fruits that tend to have a slightly sweet flavor.

Chayote is an introduced crop in India, but with indices of morphological diversity. Jain *et al.* (2017) evaluated 36 native S. edule accessions with 18 fruit-related morphological markers and 97 polymorphic bands obtained from DAMD-type markers. The PCA results are similar to those obtained by Cadena-Iñiguez *et al.* (2008), given that the main components give greater variation to the characteristics of the fruit, but not so due to the morphology in flowers and leaves, where Cadena-Iñiguez *et al.* (2008) found a variation. Verma *et al.* (2017) also found moderate genetic diversity in 74 chayote varieties in the North Eastern Hill region of India with ISSR and RAPD-like molecular markers; on the other hand, those varieties of dark green color showed the highest content of phenols and vitamin C, and are recommended for selection and hybridization.

Abdelnour & Rocha (2008) evaluated the biochemical diversity with isoenzymes in 42 *S. edule* accessions native to Costa Rica, and found that 57% of the 14 loci were polymorphic; 24 accessions were heterozygous for a single locus, and the others had polymorphisms at two or three loci. Avendaño-Arrazate *et al.* (2012) reported 59.8% polymorphism in 27 cultivated *S. edule* populations and three wild isoenzyme populations; populations of *S. edule* with dark green fruits and spines were found to have the highest observed and expected heterozygous values; even so, the observed average heterozygosity was low (0.05). The highest genetic variation was found by isoenzymatic analysis in materials with dark green fruits. Therefore, isoenzymes are currently not recommended for genetic diversity studies since their expression is regulated by the environment and the results may be distorted.

The recalcitrant nature of *S. edule* seeds prevents their timely conservation, making it difficult to obtain archaeological records that allow delving into themes related to their origin. However, through ethno-historical and linguistic techniques (Guevara *et al.*, 2014), it has been determined that the Aztecs used and cultivated chayote (Newstrom, 1991; Lira, 1996). Since it is the *Sechium* species with the highest degree of domestication in a region with high ecogeographic and cultural diversity such as Mexico, varietal complexes have been formed within the species that differ primarily in the physiological, chemical and anatomical characteristics related to the fruits (Cadena-Iñiguez & Arévalo-Galarza, 2011). The highest degree of observed heterozygosity was found in materials with an intense green color and with spines, which are prominent characteristics of wild materials (Avendaño-Arrazate *et al.*, 2012).

The fruits of the wild populations of *S. edule* tend to have a bitter flavor and high concentrations of cucurbitacins, and are rich in ethyl acetate to capture free radicals (Cadena-Iñiguez *et al.*, 2011; Riviello-Flores *et al.*, 2018). These chemical compounds have anti-neoplastic properties and apoptosis inducers in cancer cells, and can inhibit the metastatic process of lung cancer (Shukla *et al.*, 2016). The alcoholic extracts of the varietal complexes of chayote and their improved genotypes H-378 (*S. edule amarus silvestrys* × *S. edule var. virens levis* 290) and Perla negra (*S. edule var. nigrum minor* × *S. edule var. amarus silvestrys*) (Cadena-Iñiguez *et al.*, 2013a) have shown positive results in cancerous cell lines such as L929 (mice lung fibrosarcoma), HeLa (human cervical carcinoma) and P-388 (mice leukemia macrophages), with the peculiarity of having minimal negative effects on normal cells (Monroy-Vázquez *et al.*, 2009; Cadena-Iñiguez *et al.*, 2013b; Aguiñiga-Sánchez *et al.*, 2015, 2017; Salazar-Aguilar *et al.*, 2017).

The alcoholic extracts of *S. edule* decrease levels of agents related to coronary heart disease and significantly increase high-density cholesterol levels (Neeraja *et al.*, 2015). The dried fruit powder of *S. edule var. nigrum spinosum* has significant effects in reducing oxidative stress,

which helps combat metabolic syndrome problems in older adults (Rosado-Pérez *et al.*, 2019); otherwise, alcoholic extracts of *S. edule* from roots and fruits have reducing effects on blood pressure (Lombardo-Earl *et al.*, 2014; Fauziah *et al.*, 2019); they also act as regulators of blood glucose levels (Maity *et al.*, 2013; Martiar, 2017), protectors of the gastrointestinal mucosa (Sateesh *et al.*, 2012), and have antibacterial activity against the gram-negative Escherichia coli ATCC 8739, *Salmonella typhimurium* ATCC 3224 and *Shigella flexneri* ATCC 12022 (Kalpana *et al.*, 2013); just to cite some examples of its many applications.

Concerning breeding to increase the content of cucurbitacins, which confer bitter flavor both to leaves and to fruits, two genes, bi-1, and Bt-1 have been found to be involved. From a set of 2416 pairs of SSR primers developed from the sequencing of the cucumber genome (*Cucumis sativus* L.) (Ren *et al.*, 2009), Zhang *et al.* (2013) used QTL analysis to study 149 RIL´s produced by the crossing of the inbred lines 9110Gt (P1) and 9930P (P2) of cucumber to detect molecular markers linked to bitter flavor in fruits and leaves. In this way, it can possibly be detected that the *bi* (*bi-1*) and *Bt* (*Bt-1*) genes are linked and confer bitter flavour to the aforementioned structures. These genes were found on chromosomes 5 and 6 of the cucumber and could have an application to be detected in other genera of the family since it belongs to the same botanical family. The classical breeding methods, based on selection and hybridization between the species *S. compositum, S. chinantlense,* and varietal complexes of *S. edule*, have produced improved varieties and hybrids for human consumption and pharmaceutical potential due to their high content of cucurbitacin (Cadena-Iñiguez *et al.*, 2013a; Avendaño-Arrazate *et al.*, 2014).

S. compositum. Its geographic distribution is from Chiapas in Mexico to Central American countries such as Guatemala. It can develop up to an altitude of 2100 m, allowing it to grow in humid environments, such as mesophyll and tropical forests. Newstrom (1990, 1991) described it as the wild species closest to S. edule, despite having different chromosome numbers [(*S. compositum* n = 14) (Lira *et al.*, 2009) (*S. edule* n = 12, 13 and 14) (Palacios, 1987; Mercado *et al.*, 1993; De Donato & Cequea, 1994)] and because it has the singularity of not having an apical fissure, which is a characteristic that can be used as an object of selection (Lira, 1995) or isolation.

S. chinantlense. This is limited to northern Oaxaca, Mexico, with an altitude below 800 m. It develops in high or medium evergreen forests, and in mesophyll and deciduous forests. *S. chinantlense* is related to *S. edule* and *S. compositum*, which is why its conservation is necessary to understand the evolutionary processes of cultivated species (Lira, 1995; Lira *et al.*, 1999).

Taxonomic studies of palynology by Lira *et al.* (1994) showed that the qualitative physical characteristics of the pollen grains (thorny and covered grains) of *S. chinantlense* belong to the Siciynae subtribe and share certain characteristics with the rest of the *Sechium* species; however, the absence of an analysis of variance makes it difficult to observe the presence/absence of significant differences.

S. hintonii. It has a restricted distribution in Temascaltepec (Mexico State), and in the southern state of Guerrero (Mexico). It thrives in a limited altitude range, fluctuating between 1300 and 1500 m in deciduous tropical forests and live oak forests (Lira, 1995). Recently, it has been collected from the mesophyll forest of Veracruz at an altitude of 1340 m in the germplasm bank of the Grupo Interdisciplinario de Investigación en *Sechium edule* en México (GISeM).

S. mexicanum. This species is endemic to Mexico and is found in the states of Hidalgo, Puebla, Querétaro and Veracruz. It develops at altitudes of 900-2300 m in mesophyll, oak, and pine forests (Lira & Nee, 1999). Molecular studies suggest that this species should be relocated to another genus, and *Sicyos* seems to be the closest (Cross *et al.*, 2006; Sebastian *et al.*, 2012).

S. tacaco. This species is totally restricted throughout the Talamanca mountain range, Costa Rica, at altitudes of 1000-1700 m, and is locally known as "tacaco" (Wunderlin, 1976). There exist cultivated and semi-cultivated populations of this species in San José, Costa Rica, where their fruits are exploited as vegetables to make regional dishes (Lira, 1995). Morales (1994) studied the vegetative and reproductive morphology of *S. tacaco,* and found similarities with *S. edule* in the anatomical structure of plant organs, multicellular trichomes, structure of floral nectaries, and the presence of anomocytic stomata in vegetative and reproductive organs. Lira (1995) emphasized the low morphological diversity of *S. tacaco* fruits, except for the presence/absence of spines and the amount of fiber; however, in a recent study, Monge & Loría (2017) described morphologically five varieties of *S. tacaco* in geographically distant localities, although at similar average altitudes of 1100 m, thereby finding diversity and significant differences in the characteristics of the fruit (weight, length, width). The most important findings of Monge & Loría (2017) were found in the report of *tacaco* fruits with 6-7 complete longitudinal sutures and 2-5 incomplete longitudinal sutures, which provided new information to elucidate evolutionary processes at the level of species and *Sechium* genus.

S. panamense. This species is endemic to Panama, where it develops at altitudes that fluctuate between 1500 and 3000 m. Its botanical description is only based on the four samples collected, so it is necessary to intensify its search to find the variation and possible hybridizations that will contribute to its study. Its adaptation to cold climates makes it ideal to become incorporated into genetic improvement programs, which would help induce its search and research (Lira, 1995).

S. talamancense. Also known as "chayotillo" and "tacaquillo", *S. talamancensis* is endemic as *S. tacaco* to the Talamanca mountain range in Costa Rica. It is found in the cloud (mesophyll) forest at altitudes of 2400 to 3200 m. Due to the low temperatures where it thrives, it could be useful in the transfer of genes to improve frost-resistant cultivated materials (Lira, 1995).

S. venosum. This species is endemic to the Caribbean coast of Costa Rica and is very similar to *S. hintonii* in terms of its pendular inflorescences. It adapts perfectly to conditions with a high degree of moisture (Lira, 1995), suggesting that its genetic structure is resistant to attack by fungal diseases, which are the main problems in cultivated species of *Sechium* (Olguín-Hernández *et al.*, 2013) and one of the most significant approaches in the genetic improvement of species with agricultural importance (Newstrom, 1990; Moncayo-Pérez *et al.*, 2020).

S. villosum. This species is endemic to Costa Rica, and thrives in disturbed environments of tropical or mesophyll forests, at altitudes of 1500-2000 m. Similar to *S. venosum*, it develops in humid environments, which could be a source of genes for resistance to fungal-related diseases (Lira, 1995).

S. pittieri. This species is distributed from Nicaragua to Costa Rica, where it can be consumed as a vegetable. Its phenotypic and genetic plasticity allows it to adapt in diverse environments with a broad altitudinal range that covers up to 2500 m, which can be synonymous with *S. pittieri*, possibly having a wide range of genetic diversity, and can also be used as *S. talamancensis* and *S. panamensis* to improve cultivated species (Lira, 1995).

Background of taxonomic classification

Jeffrey (1978) described seven species of *Sechium* and grouped them into two sections (*Sechium* and *Frantzia*) based on the characteristics of their floral nectaries. The anthers of the *Sechium* section (*S. compositum, S. edule, S. hintonii* and *S. talamancense*) are generally free, which confers on them a plesiomorphic state, while in *Frantzia* (*S. pittieri* and *S. villosum*), the anthers are totally fused, indicating an apomorphic characteristic; this is possibly the reason for describing these two sections for *Sechium*.

Newstrom (1986, 1991) only considered three species of *Sechium* (*S. compositum, S. edule* and *S. hintonii*) and, like Jeffrey (1978), did not include *S. chinantlense* within this group. Newstrom decided to group the rest of the Central American species in the Polakowskia and *Frantzia* genera (*S. tacaco, S. talamancense, S. pittieri, S. panamense, S. venosum* and *S. villosum*); these two genera are currently found to be unaccepted or unresolved (The Plant List, 2010). In the studies by Lira & Chiang (1992), Lira (1996), Lira *et al.* (1997a,b; 1999),

Lira & Nee (1999), the *Sechium* genus was divided into the *Sechium* (*S. compositum, S. chinantlense, S. edule, S. hintonii, S. tacaco* and *S. talamancense*) and *Frantzia* (*S. mexicanum, S. panamense, S. pittieri, S. venosum* and *S. villosum*) sections. The cultivated species *S. edule* was included in the *Sechium* section, as well as the wild species closest to it (*S. compositum, S. chinantlense* and *S. hintonii*); the semi-domesticated Central American species *S. tacaco* and *S. talamancensis*, which are phylogenetically close species, were also added. Cross *et al.* (2003, 2006) only grouped in *Sechium* the species of the Mexican clade (*S. compositum, S. chinantlense, S. edule* and *S. hintonii*).

Lira *et al.* (1997b) studied ten species of *Sechium* and 11 of the Sicynae subtribe using numerical taxonomy of 62 quantitative and qualitative characters, corresponding to the palynology and general morphology of plants. In cluster analysis, the ten *Sechium* species are well differentiated from the others; however, within the *Sechium* group, no congruence was observed regarding the classification of Jeffrey (1978). *S. pittieri* and *S. villosum* (*Sechium* section *Frantzia*) are separated by a considerable Euclidean distance. In the PCA, the characteristics of the flowers and fruits were the ones with the greatest variation, and the grouping pattern does not support the classification elaborated by Newstrom (1986) (*Sechium* sensu strict without sections, *Polakowskia* 'sensu lato' and *Frantzia* strict); thus, this analysis manages to separate the *Sechium* species into two geographic groups (Mexico and Central America).

The wild populations of *S. edule* reside mainly in the states of Veracruz and Oaxaca, but with potentially suitability potential in Querétaro, Hidalgo and Puebla. Newstrom (1991) claimed that the wild population of Oaxaca type I wild chayotes was highly homogeneous in its fruit, regardless of its shape, dark green color and high density of spines; on the other hand, he named the population of Veracruz as type II wild chayotes for having phenotypic ranges in the characteristics of the aforementioned fruits. In both classifications, the fruits are bitter and fibrous. However, Lira *et al.* (1999) decided to group them as *S. edule* spp., with edule for cultivated chayotes and *S. edule* spp. sylvestris (wild chayotes types I and II), was nominated as the most probable ancestors.

Morphological markers were used in the first three classifications of the *Sechium* species. As mentioned earlier, morphological structures undergo changes through natural and artificial selection, thereby making it difficult to identify the species through the state of plesiomorphic and apomorphic traits (Kück & Wägele, 2016). The crosses and hybridizations between species of *Sechium* can also lead to taxonomic problems. However, many of the samples from the wild subspecies have a high probability of being the product of breeding with other species of the genus, which has been widely proven in the Internal Transcribed Spacer (ITS) and External Transcribed Spacer (ETS) studies by Cross *et al.* (2006), where wild

samples of *S. edule* are grouped with *S. chinantlense, S. compositum* and particularly with *S. hintonii*.

Phylogenetic inferences in *Sechium*

The phylogenetic origin of *Sechium* has several aspects, depending on the type and analysis of the data. The conclusions of Lira *et al.* (1997a) were subjected to discussion through molecular studies by Cross *et al.* (2006) when analyzing ITS and ETS sequences in 42 species of the Sicynae subtribe (*Rytidostylis carthaginesis, Microsechium helleri, Parasicyos dieterleae,* three *Sechiopsis* species, 27 *Sechium* accessions represented by *S. chinantlense, S. compositum,* the two subspecies of *S. edule, S. hintonii, S. mexicanum, S. pittieri, S. tacaco, S. talamancense, S. villosum* and nine species of *Sicyos*). The heuristic and maximum parsimony methods do not demonstrate that the Mexican species of *Sechium* are the same species (*S. chinantlense, S. compositum, S. edule, S. hintonii*); on the other hand, *S. mexicanum* was grouped with *Sicyos,* a genus that in turn is closely related to *Sechium*. These ITS and ETS sequence data do not provide sufficient information about the phylogeny of the Mexican *Sechium* species, except for the well-supported Central American species. The consensus tree of Cross *et al.* (2006) contains many polytomies, thereby making it difficult to resolve the phylogenetic relationships of *Sechium* due to the probable rapid evolution of these species. However, the molecular clock test calculated using a penalized likelihood estimation approach and fossil data from *Sicyos,* suggests that *Sechium* species diverged in the Pleistocene, and climatic fluctuations led the species to be genetically isolated and differentiated between interglacial periods.

Cross *et al.* (2006) obtained 453 Amplified Fragment Length Polymorphism (AFLP) markers for 127 individuals from *S. edule* spp. edule, 21 of *S. edule* spp. *sylvestris*, 20 from *S. chinantlense* and 10 from *S. compositum.* The philogram obtained by the neighbor-joining method suggests that the *S. edule* spp. *sylvestris* does not form a monophyletic group, which is similar to the classification made in 1986, since the geography of these accessions is related to the states of Oaxaca and Veracruz with their respective forms of cultivated chayote; for example, some AFLP were exclusive of *S. chinantlense* and populations of *S. edule* spp. *sylvestris* from Oaxaca, thus opening the possibility of gene flow between these species. Analysis of molecular variance suggests that only 7% of the variation occurs between species, 57% between populations and 36% within individuals. The high number of sampled populations may be responsible for the greater variation between populations.

The chromosome number of *S. edule* has been studied and reported by various authors, and the results of each author differ considerably. Palacios (1987) reported n = 12 for *S. edule* from Veracruz; Mercado *et al.* (1993) n = 13 for *S. edule* from Oaxaca; De Donato & Cequea (1994) report n = 14 in six variants of *S. edule*. These differences may be due to a process called dysploidy, where there are meiotic mutations that cause chromosomal translocations without losing or gaining the genome, although it can change the chromosome sets and lead to subsequent speciation events (De Storme & Mason, 2014).

Although these three species have different chromosomal loads, it seems that intraspecific hybridization between them is possible. AFLP study can partially explain the questioning of different chromosome numbers in the subspecies of *S. edule,* as proposed by Lira (1999). Dysploidy may play an important role in interspecific crosses, chromosome number, and progeny stability.

Sebastian *et al.* (2012) studied the sequences of the *rbcL* and *trnL* genes, the trnS-trnG intergenic spacers, *rpl20-rps12, psbA-trnH* and the ITS region of the ribosomal DNA in 112 representative accessions of 87 species of *Sicyoeae,* including the 11 species of *Sechium*. The objective of this work is to study the phylogeny and biogeography of the *Sicyos* species. The maximum likelihood phylogram using the GTR + Γ model, as shown by Sebastian *et al.* (2012), managed to separate the *Sechium* species into two large clades; however, like Cross *et al.* (2006), *S. mexicanum* does not cluster in either of these two clusters, and is more related to the *Sicyos* species. Despite being a molecular study, it does not agree with the taxonomic classification of Lira (1996), Lira & Nee (1999), Lira & Chiang (1992) and Lira *et al.* (1999), since *S. tacaco* and *S. talamancense* do not belong to the *Sechium* group section *Sechium*; hence, *S. mexicanum* should not be grouped with Central American species (*Sechium* section *Frantzia*). The phylogram of Sebastian *et al.* (2012) does not present polytomies and the *Sechium* sensu stricto group (*S. compositum, S. chinantlense, S. edule* and *S. hintonii*) described by Cross *et al.* (2006); in this case, *S. chinantlense* and *S. edule* represent the most recent divergence event with a million years, followed by S. compositum and S. hintonii with 1.9 and 4.9 million years, respectively; however, stronger evidence is still required to ensure that *S. compositum* or *S. hintonii* were the direct ancestors or participants in the formation of wild populations of *S. edule.* Populations of *S. chinantlense* adapted to tropical conditions were moved to conditions of higher altitude, such as pine and oak forests, which might have originated in isolated populations in mountainous regions of the states of Veracruz and Hidalgo, Mexico, and these geographic barriers aided genetic differentiation between populations of *S. edule* (Cross *et al.*, 2006).

Regarding the Central American clade, the most recent divergent species are *S. pittieri* and *S. villosum,* with approximately five hundred thousand years. The oldest species within the clade is *S. venosum,* with a history of over 7.4 million years, followed by *S. talamancensis*

and *S. panamensis* (Sebastian *et al.*, 2012). These three mentioned species adapt to the low temperatures in the mountainous zones of Costa Rica and Panamá. Although the Central American clade has hardly been studied, its species have important characteristics that can contribute to the genetic improvement of cultivated species (Lira, 1995), either through interspecific breeding or through more sophisticated methods of genetic engineering.

Molecular data can function as a tool in the taxonomic classification of species. In this context, *S. mexicanum* has problems of being grouped in the two clades of *Sechium*, thereby having more affinity with species of the genus *Sicyos* (Cross *et al.*, 2006; Sebastian *et al.*, 2012). Further studies and reassessments are needed to correctly assign and classify *S. mexicanum*. However, until the date of the divergence of the *Sechium* genus, the most ancient species of the genus has been taken as a reference; in this case, *S. mexicanum*, which began to diverge approximately 15 million years ago in the Cenozoic era (Sebastian *et al.*, 2012) (Fig. 1).

In the Mexican clade, *S. edule*, *S. compositum* and *S. chinantlense* were close to each other, indicating recent periods of divergence during the Pleistocene era, characterized by glacier periods; however, there were also inter-glacier periods when the sun's rays provided a warmer climate (Fig. 2). These thermal fluctuations triggered the species to contract and expand. During the expansion period, there was gene flow, introgression and hybridization between species, which led to great diversification (Ikeda *et al.*, 2012); during the contraction period, the species
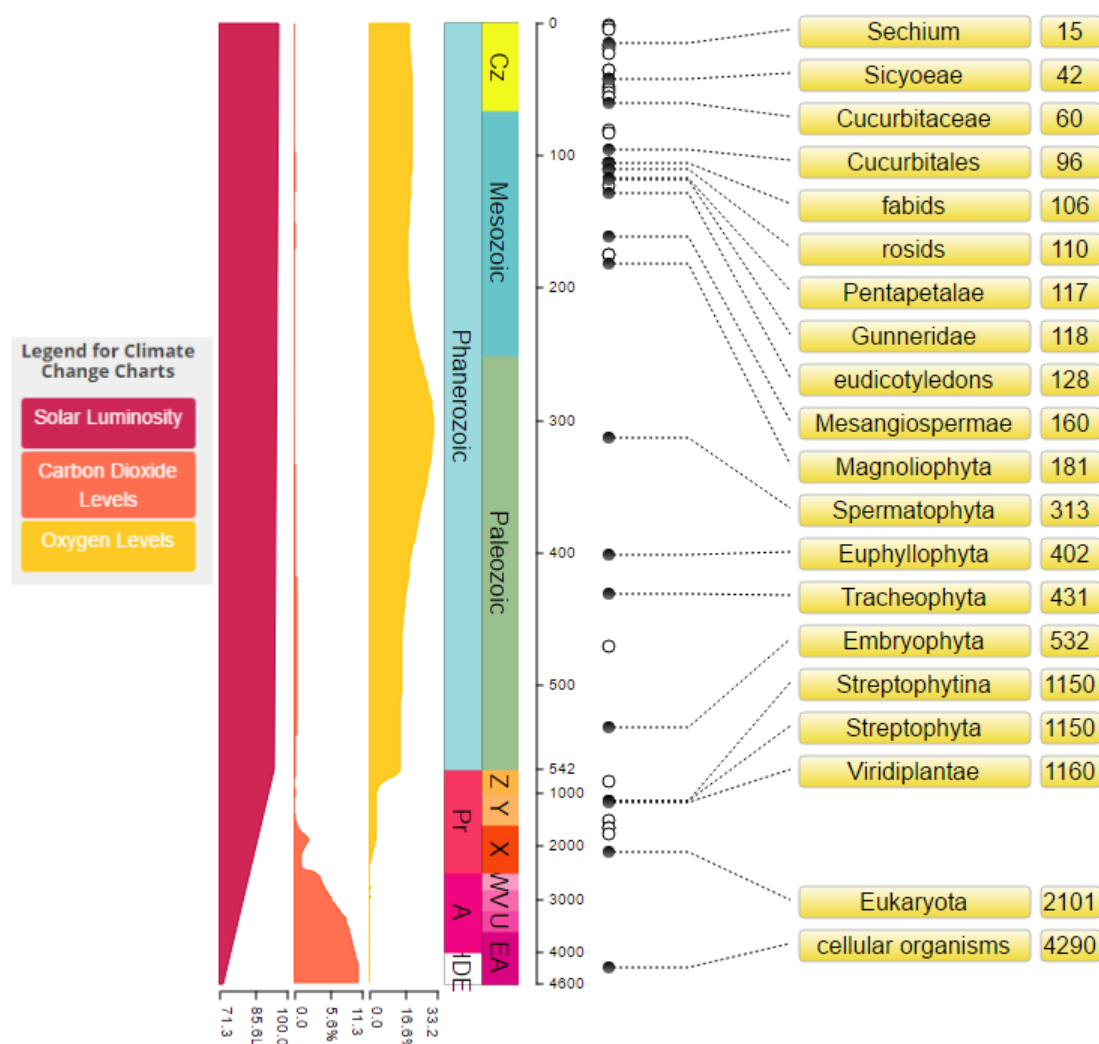

Figure 1. Evolutionary chronology for *Sechium* with divergence times in millions of years. Divergence-time estimation for all taxa in the *TimeTree of Life* (TTOL) is pre-calculated and stored in the *TimeTree* database. The evolutionary timeline shown was constructed by first locating the consulted species or superior taxa in the TTOL and then crossing the tree towards the root, thereby gathering divergence-time estimation in each node of the tree. *TimeTree* was taken from Kumar *et al.* (2017) in MEGA 7.0 (Kumar *et al.*, 2016). Ages: Cz= Cenozoic, Z= Neo-Proterozoic, Y= Meso-Proterozoic, X= Paleo-Proterozoic, W= Neoarchean, V= Mesoarchean, U= Paleoarchean, EA= Eoarchean; Eon: Pr= Proterozoic, A= Archean, HDE= Hadeon.
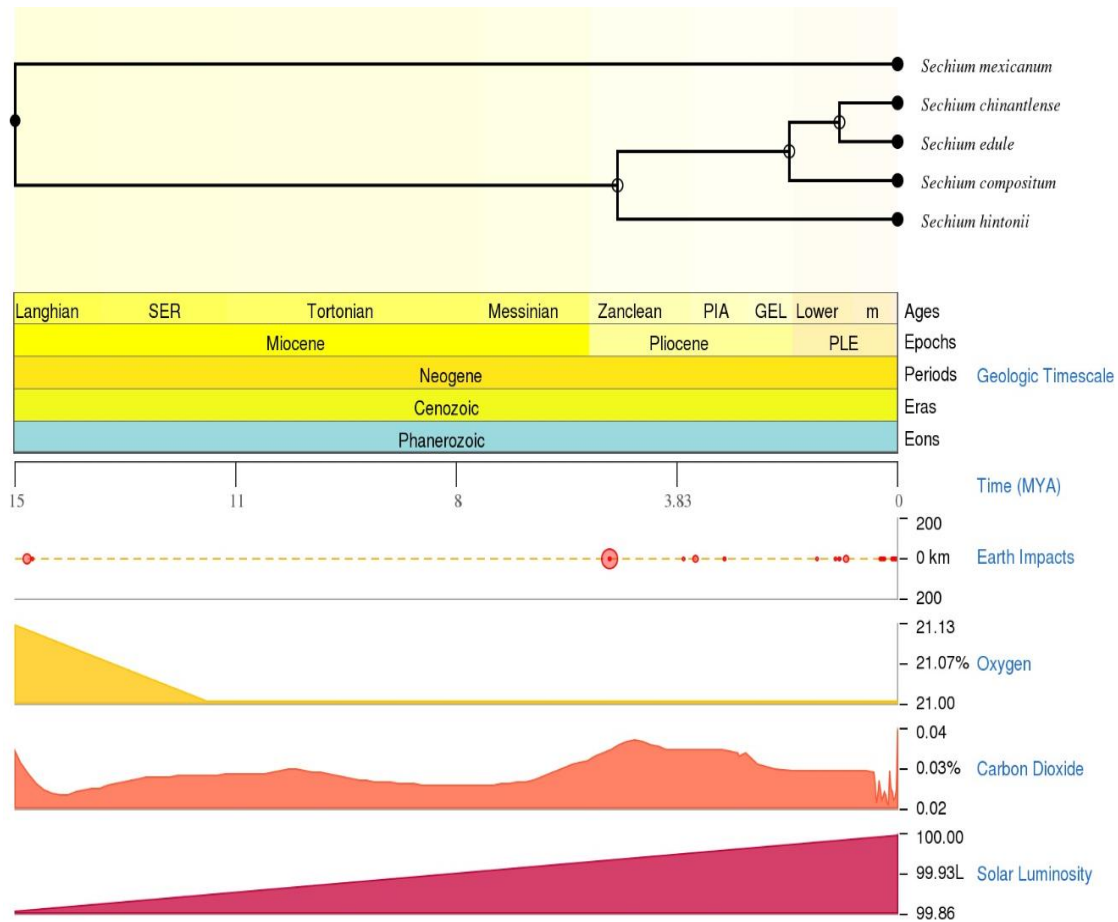
Figure 2. Evolutionary chronology of the Mexican species of *Sechium* with divergence times in millions of years. Ages: SER= Serravallian, PIA= Piacenzian, GEL= Gelasian; Epoch: PLE: Pleistocene. Elaborated with the *TimeTree* tool by Kumar *et al.* (2017) in MEGA 7.0 (Kumar *et al.*, 2016).

were isolated and this led to peripatric speciation or in situ diversification, originating from endemic species (Nevado *et al.*, 2018). In this case, *S. chinantlense* and *S. compositum* seem to have undergone hybridization with populations of *S. edule* and were then geographically isolated as they are generally found in restricted zones. The collections of Mexican chayote have been performed in mountainous regions (GISeM, 2015), which stand out for having physiographic and climatic wealth that allows the diversification of species and cause divergence through adaptive specialization, just like the emergence of varietal chayote complexes (Cadena-Iñiguez *et al.*, 2007, 2008, 2011). In the Central American clade, *S. villosum* and *S. pittieri* represent the most recent divergence event, and in contrast, *S. venosum* is considered the oldest species (Fig. 3).

The taxonomic studies of Jeffrey, Lira and Newstrom, cited in this study, were primarily based on morphological characteristics of flowers, fruits, leaves and pollen grains. Plant morphology tends to present a certain phenotypic plasticity depending on the structure of interest. Generally, the differences between cultivated and wild plants lie in their fruits, which are selected by human groups to

satisfy their needs, where the same characteristics can be established throughout the generations. Despite the different chromosome load between species of *Sechium*, intra- and inter-specific breeding has been naturally possible, which has generated different karyotypes in the wild and cultivated populations of *S. edule* (Avendaño-Arrazate *et al.*, 2014). Dysploidy may play an important role in controlling the chromosome number, thereby allowing the progeny to be stable and fertile. Natural breeding, fixation of traits, natural and artificial selections, as well as the type of samples from different geographical zones, cause changes in the taxonomic classification of *Sechium*. At the morphological level, it is very complicated to discern the origin of outbreeding or determine the predominant morphological pattern.

In the study by Cross *et al.* (2006), it is assumed that the wild populations of *S. edule* were formed by hybridization with the species *S. compositum, S. chinantlense* and *S. hintonii*. The three species are morphologically different, which would entail that wild populations would also be the product of hybridization; another explanation is that the populations of these three species were subjected to artificial selection and evolved until their fruits
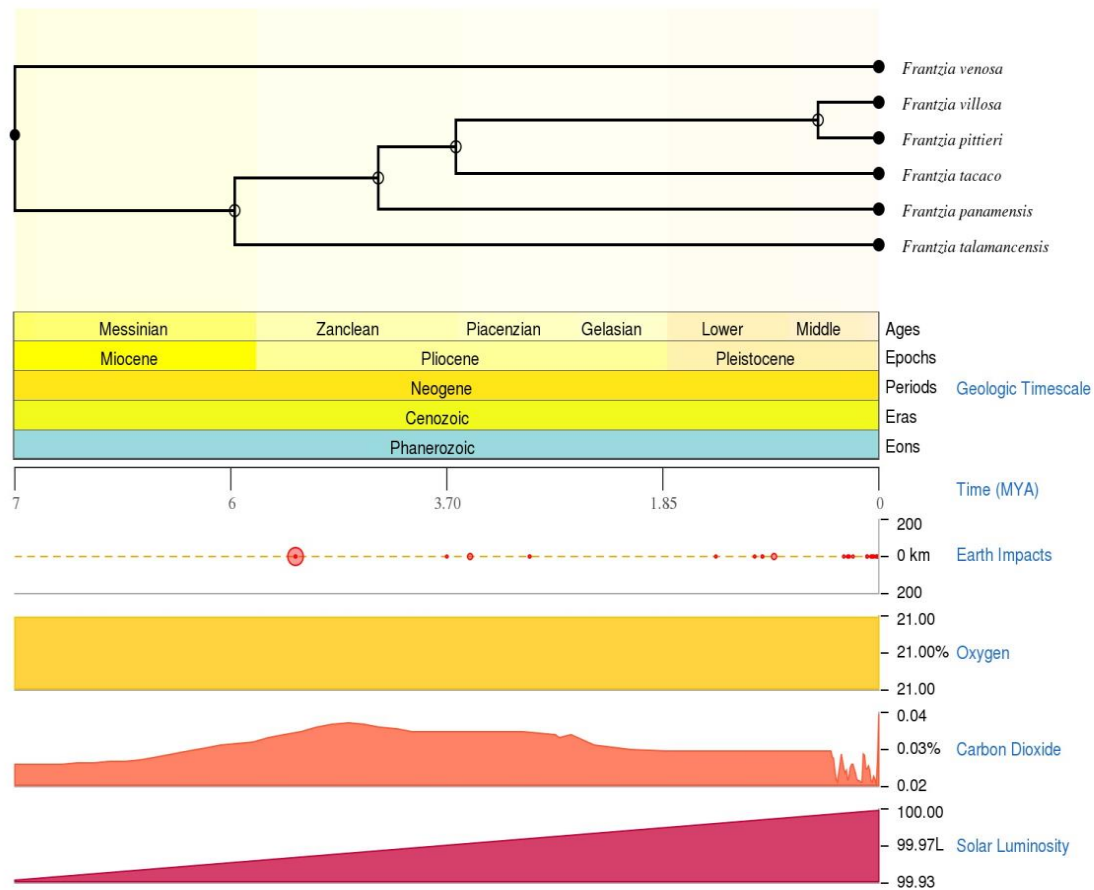
Figure 3. Evolutionary chronology of the Central American species of *Sechium* (*Frantzia*) with divergence times in millions of years (MYA). Elaborated with the *TimeTree* tool by Kumar *et al.* (2017) in MEGA 7.0 (Kumar *et al.,* 2016).

had similar morphologies. Currently, the varietal complexes of *S. edule* described by Cadena-Iñiguez *et al.* (2007, 2008, 2011) can provide answers about the origin of *S. edule*. The varietal complexes with characteristics of dark green color and the presence of spines are the closest to wild relatives; it is followed by populations with light green color and smooth texture, while the yellow varietal complexes are considered the most distant from their wild relatives through adaptive speciation to the environment, rather than due to domestication, some which have ancestral characteristics, such as the presence of spines and small fruits. The study of these varietal complexes at the level of extra-nuclear DNA can provide clues to clarify the phylogenetic origin and diversification of *S. edule*. Molecular studies have not yet fully clarified the origin and formation of the Mexican *Sechium* species; however, *S. edule* and *S. chinantlense* constitute the most recent divergence event, even so more information is needed to determine whether *S. compositum* and *S. hintonii* participated in the formation of wild and cultivated populations of *S. edule*. The speciation, gene flow, and geographic isolation events of the Mexican *Sechium* species probably occurred in the interglacial periods of the Pleistocene.

Perhaps a complete genome scan for these species can be a useful tool. The Central American clade is well supported with molecular data, but not with morphological data.

Conclusion

The differences in the taxonomic classification of *Sechium* species are the product of the wide morphological variability present between the studied samples for each species. This variation is due to the processes of natural and artificial selection, spontaneous outbreeding, geographical location of collected samples, and genetic flow between species; otherwise, the existence of plesiomorphic and apomorphic traits will also cause changes in taxonomic classification.

A reassessment is required to correctly place *S. mexicanum*, since a greater affinity was found with the genus *Sicyos* in this analysis. The new studies for *Sechium* require an integral database approach to more accurately determine phylogenetic relationships within the genus. On the other hand, the Central American species of *Sechium* have important characteristics that can be introduced into

cultivated species through classical genetic improvement and assisted by molecular markers.

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