

Determining the identity of New Zealand kamokamo (*Cucurbita pepo*, Cucurbitaceae) using mitochondrial DNA and morphological data

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Abstract

Kamokamo is a vegetable introduced to New Zealand during early European settlement that has become a prized food for Māori. Kamokamo is *Cucurbita pepo*, which includes pumpkins and squashes, but its subspecies classification was unknown. This study is the first investigation into the affinities of kamokamo, using mitochondrial *nad1* DNA sequences and morphological characters. The *nad1* sequence from kamokamo was identical to published sequences from *Cucurbita pepo* ssp. *pepo* and morphological measurements provided additional support that kamokamo belongs to this taxon. Kamokamo plants were grown from six different seed sources and within these two distinct fruit morphologies were observed.

Additional keywords: Cucurbitaceae, *Cucurbita pepo* ssp. *pepo*, kamokamo, morphology, *nad1* DNA sequences

Introduction

The Cucurbitaceae family includes many widely cultivated vegetables that have been domesticated by humans, including pumpkins, marrows, cucumbers, gourds and melons. Within the Cucurbitaceae the New World genus *Cucurbita* comprises 12-14 species and molecular data have revealed the genus has undergone at least six separate domestication events (Nee, 1990). The *Cucurbita* genus contains some of the earliest known domesticated plant species with archaeological remains of domesticated *C. pepo* dating to 10,000 years ago found in Mexico (Smith, 1997). Advances in molecular techniques have increased understanding of relationships within *Cucurbita* and provided insight into the origins of domesticated species (Sanjur

et. al., 2002). Presently three species are economically important worldwide, *C. pepo* L., *C. moschata* Duchesne and *C. maxima* Duchesne (Robinson and Decker-Walters, 1997).

Cucurbita pepo includes common vegetables such as marrows, zucchinis, pumpkins, squashes and some types of gourd. This species has been described as perhaps exhibiting the most variable fruit characteristics of any plant species (Katzir *et al.*, 2000; Paris *et al.*, 2004) with much of this diversity generated through selective breeding subsequent to its introduction to Europe in the sixteenth century (Decker, 1988). The fruit vary in shape (long or round), size (from 100 g to 20 kg), colour intensity and shading and they may have warts and/or prominent ribbing (Schaffer

and Paris, 2003). In this study the taxonomy of Decker (1988) and Decker-Walters *et al.* (1993) was followed. These authors recognized three subspecies within *C. pepo*: *C. pepo* ssp. *pepo*, *C. pepo* ssp. *fraterna* (L.H.Bailey) and *C. pepo* ssp. *ovifera* (L.). *Cucurbita pepo* ssp. *pepo* and *C. pepo* ssp. *ovifera* were domesticated from different wild ancestors (Sanjur *et al.*, 2002) and both subspecies are common in cultivation today.

Kamokamo (also known as kumikumi) is a vegetable grown in New Zealand that is thought to have been introduced during early European settlement, around the late 1800s to early 1900s (Leach, 1984). The kamokamo fruit is similar in morphology to wild forms of *C. pepo* in Mexico. Kamokamo has most likely diversified since its introduction to New Zealand (Roskrug, 2007). Deducing the path of introduction for kamokamo is difficult as *C. pepo* has been introduced multiple times to Europe since the sixteenth century (Decker, 1988). There are distinct forms of kamokamo present in New Zealand and this could have arisen from multiple origins and possibly separate introductions.

Kamokamo has become a staple food source for Māori and was quickly integrated into their yearly crop across the country via trade (Leach, 1984). The immature fruit of kamokamo is edible and it has become a vegetable valued by Māori. Mature fruit were also used by Māori as a storage vessel to hold the pulp of ripe tutu berries (Best, 1976).

Kamokamo fruit is heavily ribbed and stocky with a speckled green/white skin.

Variation in fruit shape occurs within kamokamo and has likely evolved as a result of seed selection, cross pollination and/or isolation (Roskrug and Bath, 2008). Māori prize the seed of their preferred fruit form and guard it carefully to ensure it is not affected by cross-pollination (Roskrug, 2007). Morphologically, kamokamo fruit are similar to some forms of *C. pepo* and it is currently classified as this species in the New Zealand Plant Name Database (Allan Herbarium, 2000). Andres (1995) also considered kamokamo to be *C. pepo* and placed it in the zucchini (cocozele, courgette, marrow or middle eastern) cultivar group, along with 100 other forms. However, its exact classification, including its correct subspecies designation, is not known. To confirm the species identification and determine the subspecies of kamokamo part of its mitochondrial (mtDNA) *nad1* gene was sequenced and morphological characteristics were recorded.

Materials and Methods

Sampling included a kamokamo plant obtained from a commercial nursery and specimens grown from seed obtained from five different sources (Table 1). Seeds were planted in peat pots in a mist house at the Plant Growth Unit, Massey University, Palmerston North (40°22'40"S; 175°36'27"E) and representative seedlings of each source were transplanted both into the field and glasshouse.

Table 1: Samples included for DNA sequencing and/or morphological characterisation.

Kamokamo seed name	Source	GenBank Accession	Herbarium Voucher
Nursery	Oderings Nursery, Palmerston North	GU722335	WELT SP087633
Manutuke	Gisborne	JF512563	WELT SP089728
Massey	Palmerston North	JF512565	WELT SP089726
Bulls	Manawatu	JF512566	WELT SP089725
Hanui	Hawke's Bay	JF512564	WELT SP089727
Ruaputahanga	Taranaki	JF512562	WELT SP089729

Total genomic DNA was extracted from fresh or silica-gel dried leaf tissue for one representative sample of each of the six kamokamo sources, using a CTAB extraction method (steps 1, 3-7 from Table 1 of Shepherd and McLay, 2011). The 1.6-kb region spanning intron 2 between exons B and C of the mtDNA *nad1* gene was amplified with the primers from Sanjur *et al.* (2002) and the protocol from Shaw *et al.* (2007). Each 20 μ l polymerase chain reaction (PCR) contained 1 x PCR buffer (ABgene), 1.5 mM MgCl₂, 1 M betaine, 250 μ M of each dNTP, 0.5 μ mol of each primer, 0.5 U Red Hot Taq polymerase (ABgene) and 10-100 ng of genomic DNA.

PCR products were purified by digestion with 1 U shrimp alkaline phosphatase (SAP, USB Corp., Cleveland, USA) and 5 U exonuclease I (Exo I, USB Corp., Cleveland, USA) at 37°C for 30 minutes, followed by inactivation of the enzymes at 80°C for 15 minutes. The purified PCR products were sequenced in both directions with the ABI Prism Big Dye Terminator cycle sequencing kit version 3.1 and run on a DNA sequencer (ABI 3730; Tokyo, Japan)

Kamokamo sequences were aligned manually by eye to the alignment of 12 species of Sanjur *et al.* (2002). *Cucurbita* comprises 12-14 species as there is debate around recognising some subspecies as

species. This taxonomy issue has not yet been resolved so only 12 species were aligned (noting that the other two species are included as subspecies within these species). Regions in the *nad1* alignment that could not be unambiguously aligned, as well as missing and/or ambiguous sites, were omitted prior to analyses. A maximum parsimony (MP) analysis was conducted using PAUP* (Swofford, 2002), with a heuristic search of 1000 random addition replicates and TBR branch swapping. Branch support was assessed using 1000 bootstrap pseudoreplicates, with 10 random addition replicates. Bayesian analyses (BA) were also performed, using MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001), with the default priors, nst=six, and rates set as equal as determined using the akaike information criterion (AIC) in Modeltest version 3.7 (Posada and Buckley, 2004). Two concurrent analyses were run, each with four Markov chains of 10,000,000 generations. The chains were sampled every 1000 generations, and the first 50% of these samples were discarded as 'burn-in'. At this point, the standard deviation of split frequencies was less than 0.01, indicating convergence to a stationary distribution had been achieved.

Kamokamo morphology was characterised by examining features previously used to distinguish *Cucurbita*

taxa (Table 2). Additional features from a list of descriptors for cultivated *Cucurbita* species (Ferriol *et al.*, 2009) were also recorded. Descriptors (morphological characters) include plant growth habit, peduncle shape, flower form, fruit form, prominent fruit colour, fruit skin pattern and texture, flesh colour and seed hull presence and length. Owing to unseasonal wet weather, none of the kamokamo plants had produced mature fruit by the end of this study. Therefore, only characters that could be determined for immature fruit were recorded.

Results

All six kamokamo specimens had *nad1* B/C sequences identical to those published for *C. pepo* ssp. *pepo* specimens (Sanjur *et al.*, 2002). The Bayesian phylogenetic tree representing the relationship of kamokamo to other *Cucurbita* taxa is shown in Figure 1. A herbarium voucher from each kamokamo source has been deposited at the Museum of New Zealand Te Papa Tongarewa (Table 1). Plants from four of the six kamokamo sources were used for the morphological descriptors (the Bulls and Massey seed sources did not produce large enough plants). The four sources produced

plants with very similar vegetative and flower characteristics. Distinguishing morphological character states for kamokamo and selected *Cucurbita* species are listed in Table 2 and the foliage and flowers of kamokamo are illustrated in Figure 2. Further morphological features of kamokamo include leaves of uniform colour lacking white blotches and orange-yellow flowers with short-thick conical androecia. The peduncles were roughly angled and the seeds had tan hulls. The fruit had ten ribs and the immature fruit had speckled light green and white skin with white flesh. Fruit and peduncle rib prominence and fruit shape varied between sources of kamokamo with two distinct fruit forms present. Nursery and Ruaputahanga produced elongated fruit and Manutuke and Hanui had much rounder fruit with more prominent ribbing of the fruit and peduncle (Figure 2). The level of leaf lobing and maximum number of tendril branches differed between plants from different sources. Seed shape also showed variation; seeds from Ruaputahanga were most distinct with one rounded surface and a prominent central groove on the other surface.

Table 2: Distinguishing morphological characters for selected *Cucurbita* taxa from Whitaker & Davis (1962), Merrick (1990), Paris and Nerson (2003) and Teppner (2004). Fruit characters given for kamokamo are from immature fruit.

Characteristic	Taxon						
	Kamokamo	<i>C. pepo</i> ssp. <i>pepo</i>	<i>C. pepo</i> ssp. <i>fraterna</i>	<i>C. pepo</i> ssp. <i>ovifera</i>	<i>C. argyrosperma</i>	<i>C. moschata</i>	<i>C. sororia</i>
Foliage	Moderately to deeply lobed with harsh and prickly hairs.	Moderately to deeply lobed with harsh and prickly hairs.	Deeply lobed and less prickly than those of subsp. <i>pepo</i> and subsp. <i>ovifera</i> .	Moderately to deeply lobed with harsh and prickly hairs.	Light to deeply lobed with soft, non-prickly hairs.	Shallowly lobed with soft hairs.	Lobed with stiff hairs (but less prickly than <i>C. pepo</i>).
Peduncle	Ribbed and gradually broadening towards fruit attachment.	Ribbed and gradually broadening towards fruit attachment.	Ribbed and gradually broadening towards fruit attachment.	Ribbed and gradually broadening towards fruit attachment.	Greatly enlarged by hard cork.	Smoothly grooved and flared at fruit attachment.	Thin and without thickenings.
Flowers	Triangular corolla lobes widest near to base.	Triangular corolla lobes widest near to base.	Triangular corolla lobes widest near to base.	Triangular corolla lobes widest near to base.	Corolla lobes widest at middle and rounded (except for tip).	Corolla lobes widest at middle and rounded (except for tip).	Corolla lobes widest at middle and rounded (except for tip).
Fruit	Rind with slight to heavy 1° and 2° ribs.	Rind with 1° and 2° ribs. With short insets near peduncle end of fruit.	Smooth rind. Smaller fruit than subsp. <i>pepo</i> .	Rind smooth or with interstitial ribs. Smaller fruit than subsp. <i>pepo</i> .	Smooth to slightly ribbed.	Smooth or with rounded ribs.	Smooth.
Seed length	15-19 mm	9-26 mm	9-10 mm	9-13 mm	15-35 mm	8-21 mm	7.5-11 mm

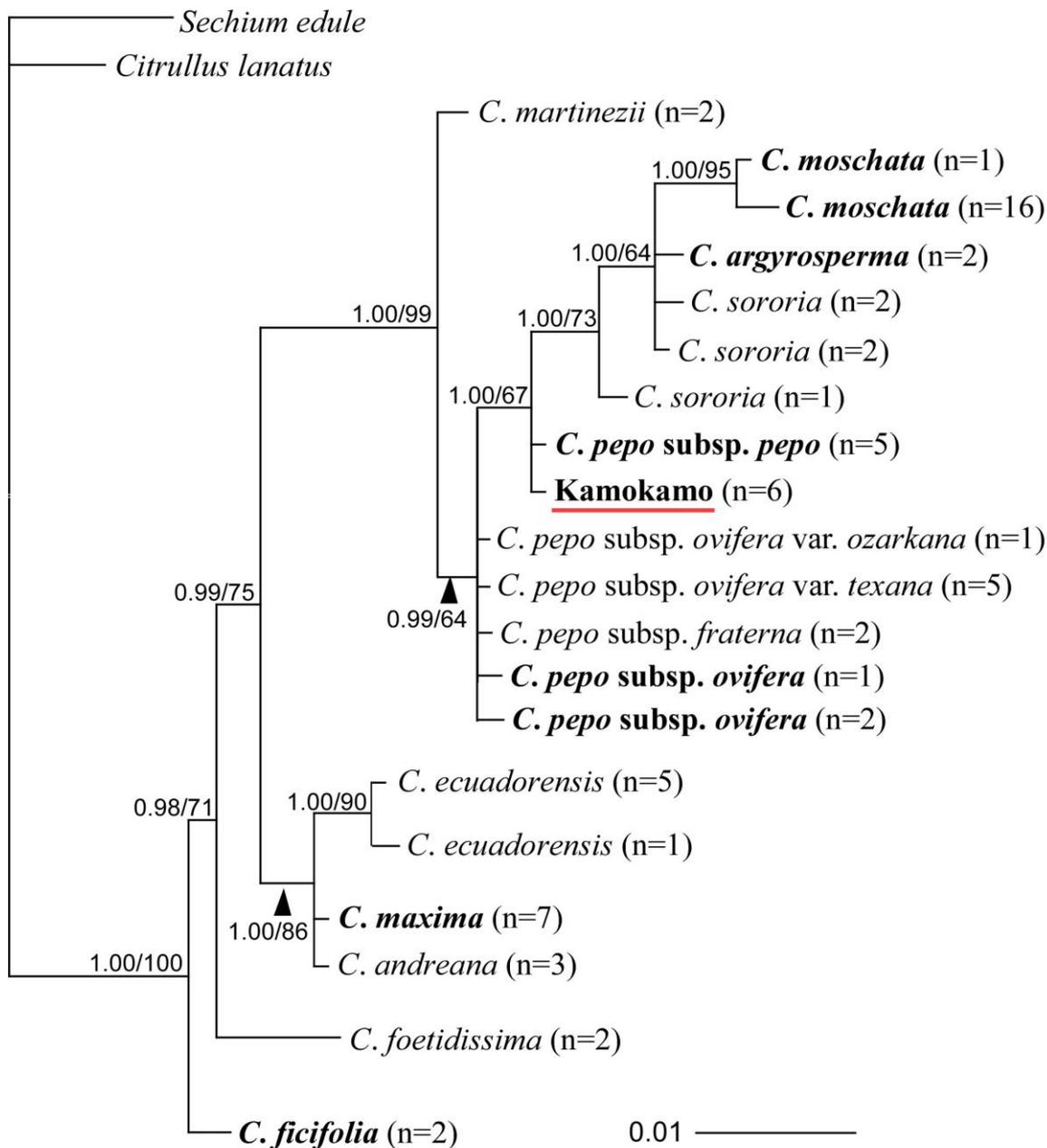


Figure 1: Bayesian phylogeny of mitochondrial *nad1* sequences from kamokamo and previously published (Sanjur *et al.*, 2002) *Cucurbita* taxa. Taxa shown in bold are domesticated. Kamokamo is underlined in red. Nodal support values are indicated (Bayesian posterior probability/maximum parsimony bootstrap).



Figure 2: (a) Kamokamo plant growing in greenhouse. Note the triangular corolla lobes of the flowers and deeply lobed leaves lacking white blotches; (b) kamokamo elongated fruit form with slight ribbing of the fruit and peduncle. The peduncle slightly widens towards the fruit attachment point; (c) kamokamo round fruit form with prominent ribs and heavily ribbed peduncle.

Discussion

The kamokamo mtDNA *nad1* B/C sequences were identical to published sequences from *C. pepo* ssp. *pepo* (from Ecuador, US, Italy, Hungary) and differed from *C. pepo* ssp. *ovifera* and *C. pepo* ssp. *fraterna* by 3 substitutions (Sanjur *et al.*, 2002). These substitutions were also shared by *C. moschata*, *C. argyrosperma* Huber and *C. sororia* L.H.Bailey. Although there

are no sequence synapomorphies exclusively uniting kamokamo to *C. pepo* ssp. *pepo*, they both lack the one synapomorphy that characterises the other species in this clade. The Bayesian phylogenetic tree representing the relationship of kamokamo to other *Cucurbita* taxa is shown in Figure 1. From the mitochondrial data it is thus likely that kamokamo is *C. pepo* ssp. *pepo*, although

additional sequence data providing synapomorphies for kamokamo and *C. pepo* ssp. *pepo* would be conclusive. The relationships between *C. pepo* and other taxa in Figure 1 have been previously discussed in Sanjur *et al.* (2002).

A limitation in this study was the use of just mitochondrial data. Mitochondrial genes have low substitution rates and high levels of gene rearrangement. However, the *nad1* intron 2, which was utilised in this study, has been used successfully in studying phylogenetic relationships in both conifers and cucurbits (Sanjur *et al.*, 2002; Gugerli *et al.*, 2001). Based on the mitochondrial data it is likely that kamokamo is *C. pepo* ssp. *pepo* but the origin is still unclear. Kamokamo could have arisen from hybridisation, between *C. pepo* ssp. *pepo* and other species or subspecies; hence we cannot exclude the possibility of kamokamo having a more complex origin than can be determined by looking at mitochondrial data alone.

Morphological data provides additional support that kamokamo is *C. pepo* rather than *C. moschata*, *C. argyrosperma* or *C. sororia*. Kamokamo and *C. pepo* share the distinguishing characters of harsh, prickly hairs on the leaves, a ribbed, non-corky peduncle, and flowers with triangular corolla lobes that attain their widest point near to the corolla tube. The seed length and presence of primary and secondary fruit ribs (but absence of interstitial ribs), combined with the molecular data, clearly indicate that kamokamo is *C. pepo* ssp. *pepo* rather than *C. pepo* ssp. *ovifera* and *C. pepo* ssp. *fraterna*.

Cucurbita pepo is underrepresented in the collections of New Zealand's herbaria, with only seven specimens recorded in the New Zealand Virtual Herbarium (New Zealand Virtual Herbarium, 2010). Furthermore,

most are not identified to subspecific level and none have been determined as *C. pepo* ssp. *pepo*. The herbarium sample deposited as part of this is the first *C. pepo* specimen for Te Papa and the first specimen identified as *C. pepo* ssp. *pepo* for New Zealand.

The morphological characterisation confirmed that two distinct fruit forms exist in kamokamo and that there is also variation in vegetative and seed characters. Measurements of mature fruit are now required to see whether any further differences, such as mature fruit skin or flesh colour, exist. Examining the morphology of more kamokamo sources may also reveal further variation. For example, some fruit have 11 ribs (R. Waihape, pers. comm., 2009). A future study could involve examining whether nuclear genetic markers show partitioning concordant with morphology. These preliminary trials have indicated polymorphism in kamokamo at four nuclear simple sequence repeat (SSR) loci (CMTp125, CMTp47, CMTp88 and CMTm11 from Gong *et al.*, 2008).

Studies into the relationships of the diverse fruit forms within overseas *C. pepo* ssp. *pepo* have found some evidence that cultivars with similar fruit shapes tend to cluster together in genetic analyses (Paris *et al.*, 2004). Inclusion of kamokamo in future such analyses may reveal whether the two fruit forms of kamokamo are each other's closest relatives or whether they are each more closely related to different overseas forms. The latter result would indicate that they had different origins and may represent separate introductions to New Zealand.

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