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Latitudinal structured populations of the Mexican wild squash *Cucurbita argyrosperma* spp. *sororia* (Cucurbitaceae) revealed by microsatellite markers

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Supplementary Table S1. Information of sampled locations of *Cucurbita. argyrosperma* spp. *sororia* in the Mexican Pacific coast

Population	State	Longitude	Latitude
San José del Progreso	Oaxaca	16.10310	-97.70497
Puerto Escondido 01	Oaxaca	15.87216	-97.09243
La Ceiba	Oaxaca	15.86229	-97.00305
Puerto Ángel	Oaxaca	15.67114	-96.48794
San José Chacapala	Oaxaca	15.82643	-96.46575
Barra de la Cruz	Oaxaca	15.83992	-95.96970
San Miguel del Puerto	Oaxaca	15.90339	-96.17575
Entrada a Puerto Ángel	Oaxaca	15.70900	-96.34095
San Andrés	Oaxaca	16.33717	-97.95173
Puerto Escondido Aeropuerto	Oaxaca	15.87527	-97.09818
Puerto Escondido 02	Oaxaca	15.87497	-97.10386
Puerto Escondido, el Tikal	Oaxaca	15.87498	-97.10401
Puerto Escondido 03	Oaxaca	15.92567	-97.12317
Puerto Ángel 2	Oaxaca	15.67741	-96.48045
Pinotepa	Oaxaca	16.37551	-98.09252
Mancuerna Pinotepa	Oaxaca	16.64817	-98.19043
Tapambo	Guerrero	18.22357	-100.87998
Entronque a Altamirano	Guerrero	17.72728	-101.60667
Barra de Potosí	Guerrero	17.57768	-101.42525
Los Laureles	Guerrero	17.34794	-101.05409
Tecpan 01	Guerrero	17.20431	-100.62937
Puente del Tejar	Guerrero	17.00385	-100.14220
San Marcos	Guerrero	16.77962	-99.39253
Cayaca	Guerrero	17.07570	-100.44387
Petatlán	Guerrero	17.91303	-101.47277
Tecpan 04	Guerrero	17.23166	-100.63162
Tecpan 02	Guerrero	17.23333	-100.62269
Tecpan 03	Guerrero	17.22567	-100.61667
Copala	Guerrero	16.60344	-98.96533
Caridad	Guerrero	16.74176	-99.29145
Cuajinicuilapa 01	Guerrero	16.79178	-98.71267
Cuajuinicuilapa 02	Guerrero	16.50332	-98.46343
Km160	Guerrero	16.63835	-98.60752
Lázaro Cárdenas	Michoacán	18.02125	-102.28100
Llanos del Bejuco	Michoacán	18.02892	-102.52692
Boca la Manzanilla	Michoacán	18.12923	-102.87578
Caleta	Michoacán	18.08092	-102.76978
Pasando Caleta	Michoacán	18.11374	-102.83905
Faro de Bucerias	Michoacán	18.35527	-103.49687
Pasando Faro de Bucerias	Michoacán	18.38645	-103.52242

Armería	Colima	18.96122	-104.04767
Tlajomulco	Jalisco	20.48517	-103.50920
Autlán	Jalisco	19.81965	-104.33925
La Huerta	Jalisco	19.49082	-104.61823
Tenacatita	Jalisco	19.32220	-104.88332
Punta Pérula	Jalisco	19.59887	-105.10815
Bahía de Banderas	Nayarit	20.77475	-105.23547
San Ignacio	Nayarit	20.84878	-105.42328
Cerro de la Cruz	Nayarit	21.52637	-104.88213
Xalisco	Nayarit	21.44668	-104.90900
Rosa Morada	Nayarit	22.11748	-105.21577
Tepic	Nayarit	21.62807	-104.96585
San Leonardo- San Leonel	Nayarit	21.38257	-104.71513
Rancho Viejo	Nayarit	21.20000	-105.08333
La Culebra	Nayarit	21.70000	-105.30000
Aticama	Nayarit	21.48477	-105.19740
Acaponeta	Nayarit	22.48273	-105.39215
Villa Unión	Sinaloa	23.19197	-106.23160
Cruz de Elota	Sinaloa	23.92055	-106.89278
Guamuchil	Sinaloa	25.18417	-107.73333
El Pozole	Sinaloa	23.18345	-106.23242

Supplementary File 1. Protocol for development and characterization of microsatellite DNA loci in *Cucurbita argyrosperma* spp. *sororia* and cross-amplification of microsatellite primers of *Cucurbita moschata*

For microsatellite development genomic DNA was digested with the restriction enzyme *Rsa*l (BioLabs) and the resulting ends of the restriction fragments were ligated with SuperSNX adaptors using T4 DNA-ligase (Promega). For the construction of microsatellite-rich libraries, digested DNA was enriched with biotinylated microsatellite oligos (Promega): (AAAC)6, (AAAG)6, (AATC)6, (AATG)6, (ACAG)6, (ACCT)6, (ACTC)6, and (ACTG)6. Hybridized fragments were captured by streptavidin-coated magnetic beads (Dynabeads M-280; Dynal) following the protocol of isolating microsatellite DNA loci (Glenn and Schable, 2005). Captured fragments were amplified using SuperSNX primers. PCR (Polymerase Chain Reaction) fragments containing microsatellites were ligated to the pGEM-T cloning vector (Promega) and transferred into competent DH5α *E. coli* cells. Recombinant colonies were grown in Luria-Bertani (LB) broth and plated onto solid LB media with 100 ppm ampicillin, IPTG 200 µg/ml, and X-gal 20 µg/ml (SIGMA-ALDRICH) for colony selection overnight at 37°C. Extraction and purification of DNA we followed the alkaline lysis protocol (Bimboim and Doly, 1979). Presence of the insert was confirmed by means of PCR using primers pUC M13 (F5'-GTAAAACGACGGCCAGT-3', R 5'GGAAACAGCTATGACCATG-3) and selecting inserts longer than 300 pb. We used the BigDye Terminator v3.0 Sequencing Kit (Applied-Biosystems) and genetic analyzer model ABI PRISM 3100 (Applied-Biosystems) for final sequencing.

Twenty four potential microsatellite loci were sequenced and analyzed with WebSat (http://purl.oclc.org/NET/websat/) for detecting microsatellite repeats. Primers were designed for 19 sequences with the bioinformatic software Primer3 using standard parameters of Primer3 system (Rozen and Skaletsky, 2000). Of these 19 pairs of primers, six showed high levels of polymorphism in the populations sampled for this study (Cucuarg11, Cucuarg04, Cucuarg13, Cucuarg02, Cucuarg07 and Cucuarg12).

Moreover, four multiplex PCR sets were optimized in order to assay 12 specific polymorphic microsatellite designed for *Cucurbita moschata* (Gong et al., 2008) in a subsample of 25 individuals of *C. argyrosperma sororia.* Overall, eight microsatellites showed amplification capacity and high levels of variation in the

investigated populations of *C. argyrosperma sororia* (CMTm7, CMTmC60, CMTm113, CMTm14, CMTm252, CMTm65, CMTmC64, CMTm83; Sanchez-Gomez 2014).