

Supporting information for

Relict inland mangrove ecosystem reveals last interglacial sea levels

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This PDF file includes:

- Supplementary Figures S1 to S5 pages 2–6
- Supplementary Tables S1 to S7 pages 7–11
- Metadata for Supplementary dataset pages 12–14

Other supplementary materials for this manuscript include the following:

- Supplementary dataset (uploaded as a spreadsheet file): Supplementary data files - San Pedro River Floristic Database.xlsx

Supplementary Figures

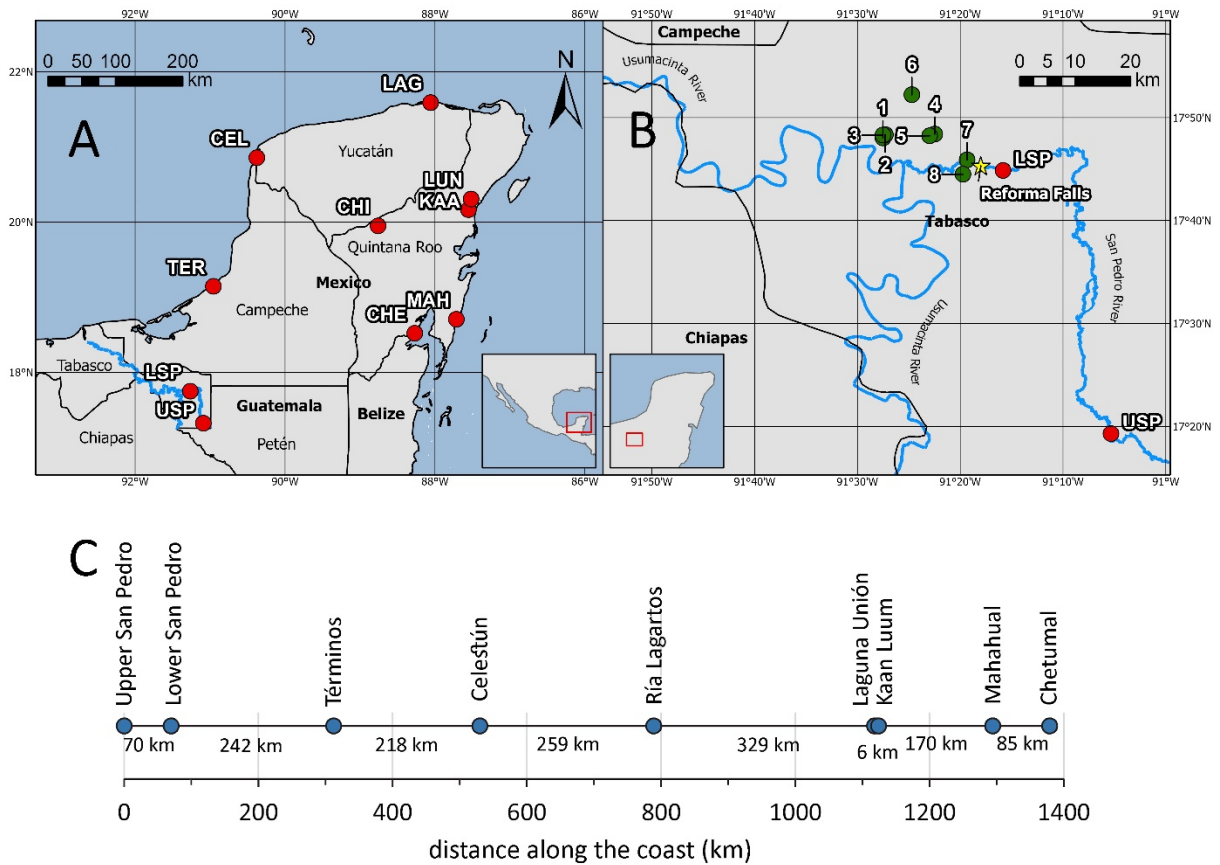


Figure S1. Location of sampling sites. **A.** Location of leaf-sampling sites in the San Pedro River and across the Yucatán Peninsula. Sampling locality symbols as follows: CEL – Celestún; CHE – Chetumal; CHI – Chichankanab; KAA – Kaan-Luum; LUN – Laguna Unión; MAH – Mahahual; LAG – Ría Lagartos; LSP – Lower San Pedro River; USP – Upper San Pedro River; TER – Términos Lagoon (see Table S2 for more details). **B.** Location of geologic sampling sites in the terraces around the lower reaches of the San Pedro River (green dots). The numbers coincide with those of the geologic observation pits reported in Table S5. **C.** Geographic distance along the coast (or along inland waterways, in the case of the San Pedro River) between nearest sites with mangroves.

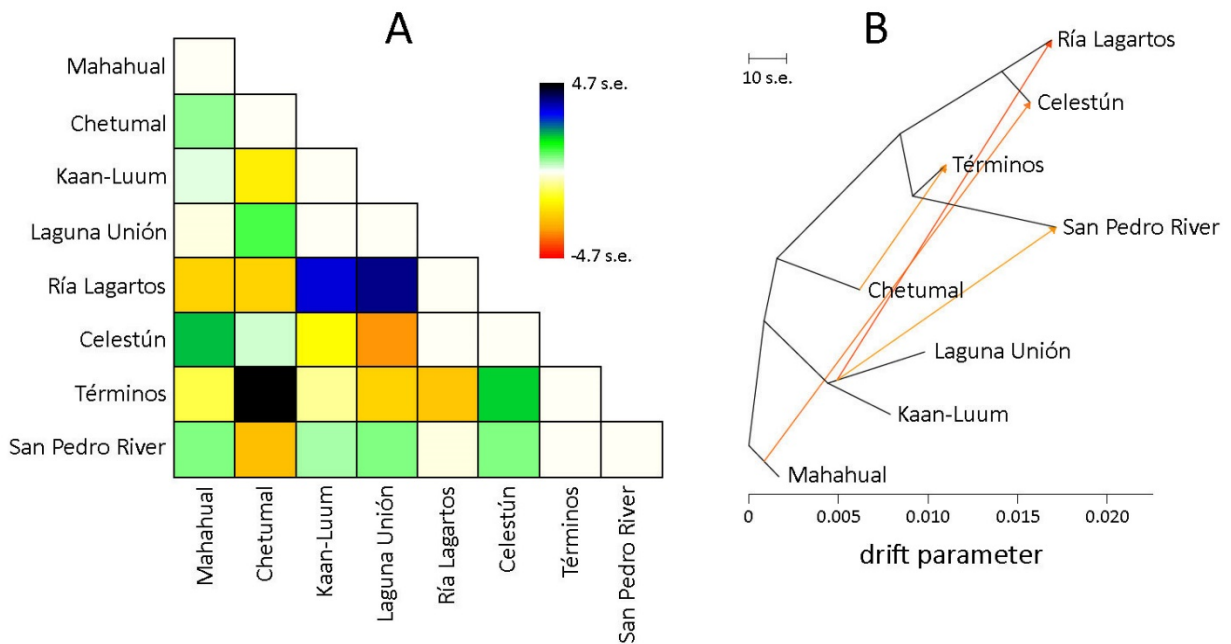


Figure S2. Pattern of genetic ancestry among *R. mangle* populations in the Yucatán peninsula inferred using TreeMix. A. Plot of residuals for the tree ancestry tree. The color palette indicates the residual covariance between each pair of populations divided by the standard error across all pairs. **B.** Best-fit maximum likelihood population tree displaying relationships between populations with four migration events. Migration edges are colored according to the proportion of source populations ancestry found in the sink population. Red indicates higher proportion, yellow indicates lower proportion.

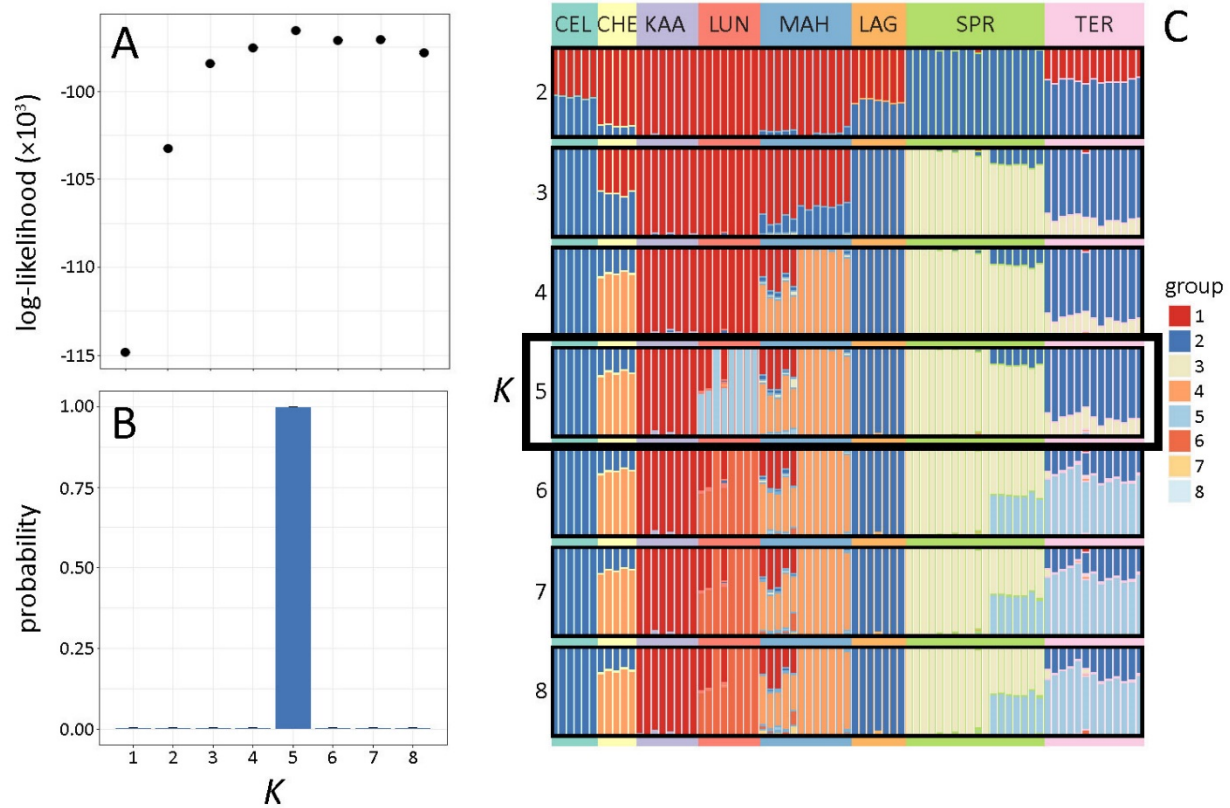


Figure S3: Pattern of genetic ancestry among *R. mangle* populations in the Yucatán peninsula inferred using Rmaverick. **A.** Weight of the evidence in support of alternative number of groups. Number of hypothesized groups (K) on the X-axis, log-likelihood on the Y-axis **B.** Posterior probability distribution of K . Number of groups (K) on the X-axis, probability on the Y-axis **C.** Posterior probability allocation of individuals to groups. Each bar corresponds to one individual, with the proportion of each color giving the posterior probability of belonging to each of the K groups. Best fit model ($K = 5$) in thick box. Sampling locality symbols as follows: CEL – Celestún; CHE – Chetumal; KAA – Kaan-Luum; LUN – Laguna Unión; MAH – Mahahual; LAG – Ría Lagartos; SPR – San Pedro River; TER – Términos Lagoon. Note the genetic distinctiveness of the San Pedro River population, and its shared similarity with the mangroves at Términos Lagoon.

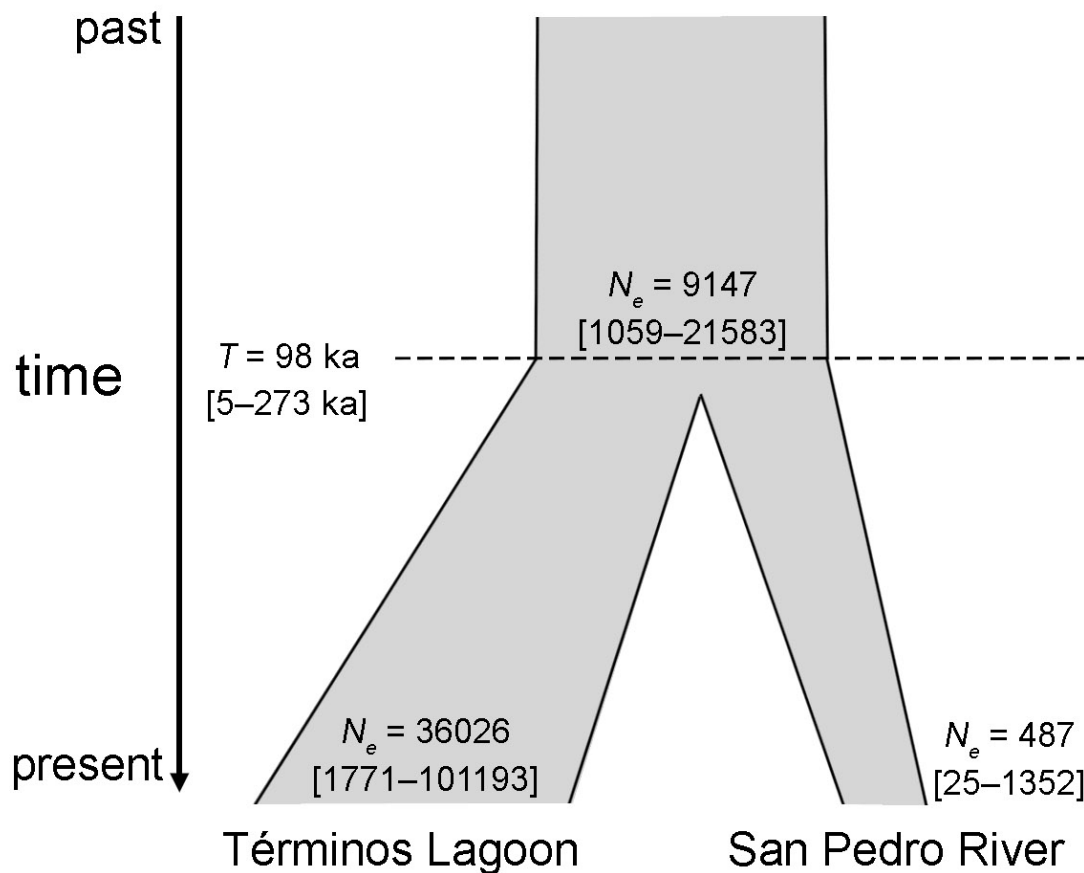


Figure S4. Inferred historical demography estimated from the SNAPP coalescent analysis. The diagram shows the median value and 95% highest posterior density interval (HPDI) for the estimate of the divergence time (T) of San Pedro River population and its closest relative in Términos Lagoon, Gulf of Mexico. The diagram's branches show the estimates of effective population size (N_e) and 95% HPDI for the Términos Lagoon population, the San Pedro River population, and the joint population before divergence. Note the dramatic reduction in effective population size at the San Pedro River after divergence, possibly as a result of isolation and inbreeding. Additionally, note that estimated range of divergence times includes the Last Interglacial (~120–130 ka BP).

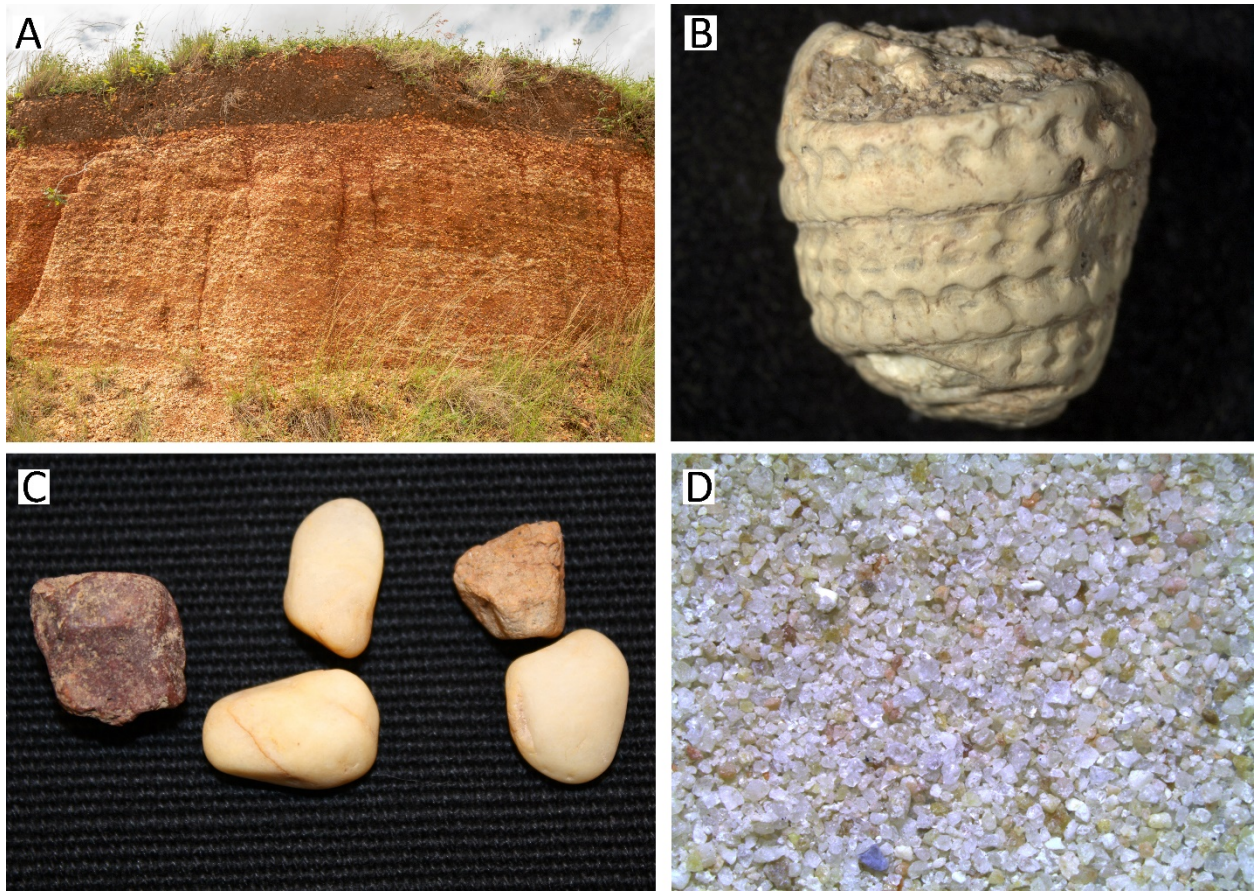


Figure S5. Geomorphology and geology of the San Pedro River. **A.** Vertical cross-section of the Pleistocene terraces at Balancán, showing the accumulation of rounded gravel. **B.** Fossil specimen of an unidentified marine gastropod found in layers of rounded gravel. **C.** Beach-eroded chert and quartz pebbles from the gravel terraces. **D.** Quartzite sand from coastal paleo-dunes.

Supplementary Tables

Table S1. Water chemistry of the lower San Pedro River at Provincia Bridge.

Chemical parameter	Sample 1	Sample 2
Ca ²⁺ (mg/L)	268.8	272.9
Mg ²⁺ (mg/L)	27.9	28.1
K ⁺ (mg/L)	0.8	0.8
Na ⁺ (mg/L)	7.7	8.0
Cl ⁻ (mg/L)	14.1	10.8
SO ₄ ²⁻ (mg/L)	796.1	809.3
HCO ₃ ²⁻ (mg/L)	79.8	79.1
pH	7.83	8.06
Conductivity (μS/cm)	1 480	1 409

Table S2. Field Site Locations.

Field Expedition 1 – July 2016

Site	Coordinates	No. of DNA extractions
Laguna de Términos I	18°59'13"N, 91°11'14"W	7
Laguna de Términos II	19°08'42"N, 90°57'18"W	6
Celestún	20°51'25"N, 90°22'36"W	6
Mahahual (coast)	18°42'17"N, 87°42'52"W	7
Mahahual (lagoon)	18°58'14"N, 87°56'03"W	6
Chetumal	18°31'11"N, 88°16'10"W	6
Chichankanab	19°54'55"N, 88°46'25"W	–
Lower San Pedro River	17°44'52"N, 91°15'50"W	7

Field Expedition 2 – May 2018

Ría Lagartos	21°33'28"N, 87°49'43"W	7
Laguna Unión	20°12'44"N, 87°32'06"W	8
Kaan Luum	20°09'54"N, 87°33'10"W	8
Chichankanab North	19°57'00"N, 88°45'34"W	–
Chichankanab South	19°50'22"N, 88°44'59"W	–
Upper San Pedro River	17°19'18"N, 91°05'20"W	11

Table S3. Age of large mangrove trees. Calibrated ^{14}C ages for six large mangrove trees in two sites (three trees per site) at the Laguna del Cacahuate, Tenosique, Tabasco, Mexico. Because the sample cores for radiocarbon the samples were taken 1 m above ground, 10 years were added to the estimated age to project the age since establishment, under the assumption that it takes ca 10 years for mangrove seedlings to establish and start forming a woody stem at a height of one meter.

site	tree	age at one-meter height (y)	diameter (cm)	diametric increase rate (mm/y)	age since establishment (y)
<i>a</i>	1	194.0	21	1.08	204.0
<i>a</i>	2	159.5	17	1.07	169.5
<i>a</i>	3	259.5	24	0.92	269.5
<i>b</i>	1	163.5	18	1.10	173.5
<i>b</i>	2	269.5	25	0.93	279.5
<i>b</i>	3	268.0	28	1.04	278.0
	mean	219.0	22.2	1.02	229.0

Table S4. Parameter sensitivity analysis of divergence time estimation. Effects of different assumptions on mutation rates (μ) and generation time values (g) on absolute divergence time (T) in thousands of years (ka).

μ	g	T (ka)	T_{min}	T_{max}
$3,16 \times 10^{-9}$	90	88	4	246
$3,16 \times 10^{-9}$	100	98	5	273
$3,16 \times 10^{-9}$	110	108	5	301
1×10^{-9}	90	278	14	777
1×10^{-9}	100	309	15	864
1×10^{-9}	110	340	17	950
5×10^{-9}	90	56	3	156
5×10^{-9}	100	62	3	173
5×10^{-9}	110	68	3	190
1×10^{-8}	90	28	1	78
1×10^{-8}	100	31	2	86
1×10^{-8}	110	34	2	95

Table S5. Geological sampling. Sampling sites in the Pleistocene terraces that surround the lower part of the San Pedro River, near Balancán, Tabasco. The elevation column shows the surface elevation of the site in meters obtained from ICESat-2 data. The depth column shows the depth at which the geologic sample was obtained.

site	coordinates	elevation	depth	type	notes
1	17°48'02"N 91°27'31"W	8	2	open quarry	Conglomerate of polymictic, smooth rounded gravel, 1–5 cm in diameter, embedded in matrix of reddish sandy-loam.
2	17°48'21"N 91°27'17"W	8	1.5	open quarry	Conglomerate of polymictic, smooth rounded gravel, 1–5 cm in diameter, embedded in matrix of reddish sandy-loam.
3	17°48'18"N 91°27'31"W	12	3	open quarry	Conglomerate of polymictic, smooth rounded gravel, 1–5 cm in diameter, embedded in matrix of reddish sandy-loam.
4	17°48'24"N 91°22'28"W	18	1	water hole	Quartz sand, partially cemented by calcium carbonate.
5	17°48'14"N 91°22'58"W	13	3	open quarry	Conglomerate of polymictic, smooth rounded gravel, 1–5 cm in diameter, embedded in matrix of reddish sandy-loam. Beach-eroded gastropod shells present (cfr. family Buccinidae).
6	17°52'13"N 91°24'42"W	31	1.5	soil pit	Loose, non-consolidated sand, dominated by quartz particles. The soil horizons become cemented by calcite below 50 cm deep
7	17°46'05"N 91°18'47"W	14	4	dugout well 5 m deep	Very fine whitish-gray clay of limestone origin with calcite shell fragments and whole oyster shells (depth ca. 3–4 m), overlaid by a layer of black-organic calcite sediment, and limestone sediments above.
8	17°44'27"N 91°19'36"W	39	3	open quarry	Conglomerate of polymictic, smooth rounded gravel, 1–5 cm in diameter, embedded in matrix of reddish sandy-loam.

Table S6. Sensitivity analysis for filtering thresholds. The table shows the pairwise correlation (r^2 values) between the first and second axes of a Principal Component Analyses on each of 12 matrices with different filtering thresholds. The row and column headers show, first, the filter threshold for SNPs, and, secondly, the threshold for individuals. The number in bold show the r^2 values for the 80-75 filter, which we used in the final analysis. Note that none of the r^2 values is lower than 0.99, indicating that the first and second PCA axis of the matrix we finally used have more than 99% similarity with all other filtering alternatives. Methodological details are provided as a note to the table.

		PCA Axis 1 – r^2 values										
		60-50	60-75	70-25	70-50	70-75	80-25	80-50	80-75	90-25	90-50	90-75
60-25		1.000	1.000	1.000	1.000	1.000	0.999	0.999	0.999	0.995	0.995	0.996
60-50			1.000	1.000	1.000	1.000	0.997	0.997	0.998	0.990	0.990	0.992
60-75				0.999	0.999	1.000	0.998	0.998	0.998	0.995	0.995	0.995
70-25					1.000	1.000	0.999	0.999	0.999	0.993	0.993	0.994
70-50						1.000	0.999	0.999	0.999	0.993	0.993	0.994
70-75							0.999	0.999	0.999	0.994	0.994	0.994
80-25								1.000	1.000	0.996	0.996	0.997
80-50									1.000	0.996	0.996	0.997
80-75										0.997	0.997	0.997
90-25											1.000	1.000
90-50												1.000

		PCA Axis 2 – r^2 values										
		60-50	60-75	70-25	70-50	70-75	80-25	80-50	80-75	90-25	90-50	90-75
60-25		1.000	0.999	0.999	0.999	0.999	0.996	0.996	0.997	0.988	0.988	0.990
60-50			0.998	0.999	0.999	0.999	0.993	0.993	0.993	0.976	0.976	0.980
60-75				0.996	0.996	0.997	0.988	0.988	0.990	0.977	0.977	0.979
70-25					1.000	1.000	0.996	0.996	0.997	0.982	0.982	0.985
70-50						1.000	0.996	0.996	0.997	0.982	0.982	0.985
70-75							0.996	0.996	0.996	0.983	0.983	0.985
80-25								1.000	1.000	0.991	0.991	0.993
80-50									1.000	0.991	0.991	0.993
80-75										0.991	0.991	0.993
90-25											1.000	1.000
90-50												1.000

Methodological details. In order to test the sensitivity of our analysis to the choice of filtering thresholds, we built 12 datasets with different filtering thresholds. First, from the full data assembly out of iPyrad (51,634 SNPs), we created 4 matrices so that each SNP was represented in at least 90%, 80%, 70%, and 60% of the individuals and filtering-out the rest. After filtering SNPs with the four thresholds described, we then filtered-out in each of the four resulting matrices individuals with substantial amounts of missing data, dividing the first four matrices into three new ones, where each individual was represented in at least 75%, 50%, and 25% of the SNPs. Thus, we obtained 12 data matrices from 4 SNP-filtering thresholds \times 3 individual-filtering thresholds. In each of the resulting matrices, we used only one SNP per locus by discarding all but the SNP closest to the center in each locus (to minimize the effects of linkage disequilibrium) and retained only biallelic loci. We then ran a Principal Component Analyses (PCA) on each of the 12 matrices, and calculated pairwise correlations among axis 1 of all PCAs, obtaining a correlation matrix among the different analyses. Using a t -test, we then tested the r -values for a significant departure from the expected value of $r=1$, under the null hypothesis that filtering was having no significant effect on the PCA results. A similar analysis was done for axis 2 of the 12 PCAs. No significant departures were found between the observed correlations and the expected value of $r=1$.

Table S7. Sensitivity analysis for minimum allele frequency (MAF). The table shows the pairwise correlation (r^2 values) between the first and second axes of a Principal Component Analyses on each of three matrices with different filtering thresholds for minimum allele frequency (MAF). For this analysis, we used the 80-75-filtered data described in Table S6, and tried three different thresholds for minimum allele frequency (MAF) used to include each SNP in the analysis: MAF=0.01, MAF=0.03, and MAF=0.05. In all three cases, alleles present in a frequency lower than the chosen MAF threshold were discarded from the analysis. The correlation procedures and hypothesis tests for the sensitivity of MAF thresholds were similar to those described in Table S6. The row and column headers show the MAF thresholds for each of the correlated variables. No significant departures were found between the observed correlations and the expected value of $r=1$, indicating that the first and second PCA axis of the matrices for different MAF thresholds do not differ.

PCA Axis 1 – r^2 values

	003	005
001	0.998	0.998
003		0.999

PCA Axis 2 – r^2 values

	003	005
001	0.988	0.988
003		0.998

Metadata for list of vascular plant species from San Pedro Mártir River, Tabasco, Mexico.

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Field methods for plant collection

The principal channel of the San Pedro Mártir River (SPMR) was traversed from the Mexico-Guatemala border in the municipality of Tenosique, to the convergence with the Usumacinta River in the municipality of Balancán, by water. Reconnaissance on foot was also conducted in sites where the conditions allowed, and for this the river was divided into five sections: (1) Border of Guatemala-La Palma, (2) La Palma-San Pedro, (3) San Pedro-Provincia, (4) Provincia-Cascadas de Reforma and (5) Cascadas de Reforma-convergence with the Usumacinta River. Three or four exploratory trips were made in each section of the river, with alternate visits in the 15 months of monitoring between 2014 and 2015. These explorations included the following lagoons, which are associated to the San Pedro River: "Aldan" (17°44'25.7"N, 91° 24' 8.6"W), "Lingle" (17°45'34.3"N 91°, 24' 10.6" W), "Negra" (17°43'17.4"N, 91°23'11.5" W) & "Susil" (17°44'57.9"N, 91°23'5.4"W), the drainages: "Capulín" (17°46'16.34"N, 91°06'32.04"W) and "López Zamora" (17°47'20.68"N, 91°05' 45.09"W) and the arroyo "Sayá" (17°46'24.16"N, 91°18'18.89"W).

Botanical collection followed the techniques recommended by Lot & Chiang (1) for collection and preparation of herbarium specimens. Plants that grow on the shore of the river and lagoons up to 50 m away from the water were collected, including those that were rooted or floating in the water. In the vast majority of cases the specimens were fertile, a few infertile individuals were collected alive (e.g., orchids, bromeliads, cacti and arums) which were cultivated at the Jardín Botánico José N. Rovirosa of the Academic Division of Biological Sciences of the Universidad Juárez Autónoma de Tabasco (UJAT) until flowering, at which time they were identified and made into specimens. Specimen identification was conducted at the UJAT Herbarium by the authors, with the help of specialized tropical floristics works such as: *Flora de Guatemala* (2), *Orchids of Guatemala and Belize* (3), *Epifitas de Veracruz* (4), *The Pteridophytes of Mexico* (5), *Árboles Tropicales de México* (6), *Descripción de las especies de árboles más comunes de la sierra de Tenosique, Tabasco* (7) and the *Flora Leñosa Útil de la Sierra de Tenosique*,

Tabasco, México (8), among others. In cases of difficult or doubtful identification we relied on the assistance of specialists, primarily in the families: Bromeliaceae, Cucurbitaceae, Cicadaceae, Fabaceae, Lauraceae, Malpighiaceae, Orchidaceae and Sapindaceae. The specimens collected were deposited at the UJAT Herbarium and their duplicates will be distributed to the herbaria CICY, XAL, and MEXU. Included in the list of specimens are those collected in the study area by other researchers and deposited in the herbaria of UJAT and MEXU, as well as those mentioned in the online databases of the herbarium F, MO and K and the species listed by Lundell (9) deposited in the WIS herbarium. Nomenclature follows *The Plant List* (<http://www.theplantlist.org>, accessed in March 2020). For the elaboration of the final list the classifications of Mickel y Smith (4) were followed for the Pteridophytes, and of Angiosperm Phylogeny Group III (10) for flowering plants. Plants were classified according to their predominant habitat into (a) coastal (i.e., plants chiefly found and collected in coastal ecosystems) and (b) inland (i.e., plants predominantly found in the regional rainforests but rarely seen inland). Additionally, all species were classified according to their life-form into (a) aquatic, (b) herb, (c) shrub, (d) tree, (e) epiphyte, (f) vine, (g) liana, and (h) palm.

Analysis

In the San Pedro Mártir River 358 species in 250 genera were identified. They belong to 87 families, of Monilophytes (ferns and fern allies), Gymnosperms, and Angiosperms (Basal Angiosperms, Magnolids, Monocots and Eudicots; see floristic database). The six families with the greatest number of species are: Fabaceae (33 species, 21 genera), Orchidaceae (31 species, 23 genera), Rubiaceae (17 species, 14 genera), Malvaceae (18 species, 14 genera), Cyperaceae (16 species, 6 genera) y Bromeliaceae (16 species, 5 genera). Trees and herbs are the dominant life-forms, with 27.9% and 27.1% of the total flora, respectively, followed by epiphytes (15.4%) and shrubs (14.2%). Despite the fact that the San Pedro River is an inland waterway, in the heart the El Petén and Tenosique rainforests, almost one third (31.3%) of the species along the riverbanks and nearby ecosystems were recognized as predominantly coastal in their distribution. For more detailed information, see the "Statistics" tab on this spreadsheet.

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