

Table S1.

Tested Population	Location	Drainage Basin	Coordinates	
			North	West
San Marcos (C101)	Texas, USA	Guadalupe River	29°51'25.83	97°53'47.96
Comal Spring	Texas, USA	Guadalupe River	29°42'46.82	98°8'8.25
Weslaco	Texas, USA	Río Grande	26°7'14.52	97°57'41.44
Río Purificación, Barretal (III/9)	Mexico	Río Pánuco	24°4'42.85	99°7'21.76
Río Purificación, Nuevo Padilla (VI/17)	Mexico	Río Pánuco	24°02'35.59	98°54'15.98
San Ignacio	Mexico	Río San Fernando	24°51'53.2	99°20'02.7

Table S1. Population origins. This table shows the test population origins across the range of *P. formosa*, indicating the location, drainage basin and the coordinates of the original population collection site). There were 2 populations from the northern range (San Marcos (C101) and Comal Spring), 2 populations from the midpoint (Weslaco and San Ignacio), and 2 populations from the southern range (Río Purificación, Barretal (III/9) and Río Purificación, Nuevo Padilla (VI/17). Note: the populations sampled covered the span of the geographical distribution of *P. formosa*.

Table S2.

Locus	Characteristics in <i>Poecilia formosa</i>							
	Population	<i>n</i>	Allele No.	Allele size	H_O	H_E	<i>P</i>	F_{ST}
GA-II41 Genbank# AJ810469	Co101	5	2	118 - 126	0.800	0.533	0.429	0.083
	3VI/17	11	2	118 - 126	1.000	0.524	0.003	
	4III/9	12	2	118 - 126	1.000	0.522	0.002	
	W5-Weslaco	7	2	118 - 126	1.000	0.538	0.037	
	6SI-SanIgnacio	5	2	118 - 126	1.000	0.556	0.126	
	7aCS-7aComalSpring	11	2	118 - 126	1.000	0.524	0.003	
	8bCS-8bComalSpring	11	2	118 - 126	1.000	0.524	0.003	
GA-I47A Genbank# AJ810468	Co101	4	2	133 - 155	1.000	0.571	0.314	0.240
	3VI/17	11	2	133 - 155	1.000	0.524	0.003	
	4III/9	12	2	133 - 155	1.000	0.522	0.002	
	W5-Weslaco	7	2	133 - 159	1.000	0.538	0.037	
	6SI-SanIgnacio	5	2	133 - 155	1.000	0.556	0.128	
	7aCS-7aComalSpring	11	2	133 - 155	1.000	0.524	0.003	
	8bCS-8bComalSpring	11	2	133 - 155	1.000	0.524	0.003	
GT-II33 Genbank# AJ810474	Co101	5	2	178 - 182	0.800	0.533	0.429	0.397
	3VI/17	11	1	182	0.000	0.000	-	
	4III/9	12	1	182	0.000	0.000	-	
	W5-Weslaco	7	1	182	0.000	0.000	-	
	6SI-SanIgnacio	5	1	182	0.000	0.000	-	
	7aCS-7aComalSpring	11	1	182	0.000	0.000	-	
	8bCS-8bComalSpring	11	2	178 - 182	1.000	0.524	0.003	
GA-V18 Genbank# AJ810470	Co101	5	3	122 - 148	1.000	0.644	0.176	0.221
	3VI/17	11	2	122 - 148	1.000	0.524	0.003	
	4III/9	12	2	122 - 148	1.000	0.522	0.002	
	W5-Weslaco	7	2	122 - 148	1.000	0.538	0.037	
	6SI-SanIgnacio	5	2	122 - 144	1.000	0.556	0.127	
	7aCS-7aComalSpring	11	2	122 - 144	1.000	0.524	0.003	
	8bCS-8bComalSpring	11	3	122 - 148	1.000	0.602	0.006	
GA-I26 Genbank# AJ810456	Co101	4	2	160 - 194	1.000	0.571	0.314	0.201
	3VI/17	11	2	160 - 194	1.000	0.524	0.003	
	4III/9	12	2	160 - 194	1.000	0.522	0.002	
	W5-Weslaco	7	2	160 - 194	1.000	0.538	0.038	
	6SI-SanIgnacio	5	2	160 - 194	1.000	0.556	0.126	
	7aCS-7aComalSpring	11	2	160 - 194	1.000	0.524	0.003	
	8bCS-8bComalSpring	11	2	160 - 194	1.000	0.524	0.003	

GA-III28 Genbank# AJ810459	Co101	4	2	215 - 241	1.000	0.571	0.315	0.303
	3VI/17	11	2	215 - 241	1.000	0.524	0.003	
	4III/9	12	2	215 - 241	1.000	0.522	0.002	
	W5-Weslaco	7	2	237 - 241	1.000	0.538	0.038	
	6SI-SanIgnacio	5	2	215 - 241	1.000	0.556	0.126	
	7aCS-7aComalSpring	11	2	215 - 241	1.000	0.524	0.003	
	8bCS-8bComalSpring	11	2	215 - 241	1.000	0.524	0.003	
GA-III29B Genbank# AJ810460	Co101	5	2	255 - 257	0.000	0.356	0.111	0.889
	3VI/17	11	2	255 - 265	0.000	0.173	0.047	
	4III/9	12	1	255	0.083	0.083	1.000	
	W5-Weslaco	7	1	261	0.000	0.000	-	
	6SI-SanIgnacio	5	1	257	0.000	0.000	-	
	7aCS-7aComalSpring	11	1	257	0.000	0.000	-	
	8bCS-8bComalSpring	11	1	257	0.000	0.000	-	
GT-I41 Genbank# AJ810472	Co101	5	1	148	0.000	0.000	-	-
	3VI/17	11	1	148	0.000	0.000	-	
	4III/9	12	1	148	0.000	0.000	-	
	W5-Weslaco	7	1	148	0.000	0.000	-	
	6SI-SanIgnacio	5	1	148	0.000	0.000	-	
	7aCS-7aComalSpring	11	1	148	0.000	0.000	-	
	8bCS-8bComalSpring	11	1	148	0.000	0.000	-	
GA-IV42 Genbank# AJ810462	Co101	5	3	198 - 204	1.000	0.644	0.175	0.316
	3VI/17	11	2	198 - 202	1.000	0.524	0.003	
	4III/9	12	2	198 - 202	1.000	0.522	0.002	
	W5-Weslaco	7	2	198 - 202	1.000	0.538	0.038	
	6SI-SanIgnacio	5	2	198 - 204	1.000	0.556	0.127	
	7aCS-7aComalSpring	11	2	198 - 204	1.000	0.524	0.003	
	8bCS-8bComalSpring	11	2	198 - 204	1.000	0.524	0.003	
GA-I29B Genbank# AJ810458	Co101	4	2	229 - 255	1.000	0.571	0.314	0.228
	3VI/17	11	2	229 - 255	1.000	0.524	0.003	
	4III/9	12	2	229 - 255	1.000	0.522	0.002	
	W5-Weslaco	7	2	229 - 257	1.000	0.538	0.037	
	6SI-SanIgnacio	5	2	229 - 255	1.000	0.556	0.127	
	7aCS-7aComalSpring	11	2	229 - 255	1.000	0.524	0.003	
	8bCS-8bComalSpring	11	2	229 - 255	1.000	0.524	0.003	
GT-II49 Genbank# AJ810466	Co101	5	2	342 - 382	1.000	0.556	0.128	0.219
	3VI/17	11	2	342 - 382	1.000	0.524	0.003	
	4III/9	12	2	342 - 382	1.000	0.522	0.002	
	W5-Weslaco	7	2	342 - 382	1.000	0.538	0.037	
	6SI-SanIgnacio	5	2	342 - 382	1.000	0.556	0.126	

	7aCS-7aComalSpring	11	2	342 - 382	1.000	0.524	0.003	
	8bCS-8bComalSpring	11	2	342 - 382	1.000	0.524	0.003	
GA-III49A NEU	Co101	5	3	394 - 412	1.000	0.644	0.174	0.181
Genbank#	3VI/17	11	2	394 - 408	1.000	0.524	0.003	
AJ810461	4III/9	12	2	394 - 408	0.917	0.518	0.014	
	W5-Weslaco	7	2	394 - 414	1.000	0.538	0.037	
	6SI-SanIgnacio	5	2	394 - 408	1.000	0.556	0.127	
	7aCS-7aComalSpring	11	2	394 - 408	1.000	0.524	0.003	
	8bCS-8bComalSpring	11	2	394 - 408	1.000	0.524	0.003	

Table S2. Characteristics of the 12 microsatellites. Here, summary statistics of the 12 microsatellites that were used in differentiating the 7 different clonal lineages of *P. formosa* are shown, i.e., population, sample size, the number of alleles, the observed heterozygosity of the current generation (H_0), the expected heterozygosity (H_E), the probability of Hardy-Weinberg-Equilibrium (HWE), i.e., $H_0 = H_E$ (P), and the F_{ST} value of all populations at that particular locus. Note that loci are generally expected not to be in HWE in Amazon mollies, due to the lack of sexual recombination.

Table S3.

	Co101	3VI/17	4III/9	W5- Weslaco	6SI- SanIgnacio	7aCS- 7aComalSpring	8bCS- 8bComalSpring
Co101	-	0.148**	0.157**	0.248**	0.000	0.000	0.000
3VI/17	0.00040**	-	0.000	0.251***	0.210**	0.232***	0.244***
4III/9	0.00060**	1.00000	-	0.258***	0.221**	0.241***	0.253***
W5-Weslaco	0.00085**	<0.00001***	<0.00001***	-	0.298**	0.318***	0.320***
6SI-SanIgnacio	0.00970*	<0.00001***	<0.00001***	<0.00001***	-	0.000	0.000
7aCS- 7aComalSpring	0.00045**	<0.00001***	<0.00001***	<0.00001***	1.00000	-	0.006***
8bCS- 8bComalSpring	0.39495	<0.00001***	<0.00001***	<0.00001***	0.00035**	<0.00001***	-

Table S3. Genetic divergence. Genetic divergence among the 7 clonal lineages of *P. formosa*.

Above the diagonal are the F_{ST} values and below the diagonal are the P -values from the Markov Chain Monte Carlo exact test. Statistical significance after sequential Bonferroni correction for multiple pairwise comparisons is indicated at an experiment-wise error rate α (* $\alpha=0.05$;

** $\alpha=0.01$; *** $\alpha=0.001$).

Table S4.

	C101	VI/17	III/9	Weslaco	San Ignacio	Comal Spring 7a	Comal Spring 8b
C101	-	654.1	653.9	415.5	573	28.1	28.1
VI/17	6	-	22.51	249.8	100.4	635	635
III/9	6	1	-	255.4	90	634.1	634.1
Weslaco	8	6	6	-	196.2	399.8	399.8
San Ignacio	4	4	4	6	-	552.8	552.8
Comal Spring 7a	4	4	4	6	0	-	0
Comal Spring 8b	3	5	5	7	2	2	-

Table S4. Loci difference and geographic range. This table shows the geographical distance (km) between the 7 different clonal lineages above the diagonal. Below the diagonal are the number of loci that are different in their allelic pattern between the different clonal lineages. Interestingly, the San Ignacio clonal lineages is located 552.8 km south of Comal Spring, yet the Comal Spring 7a lineage is identical to the San Ignacio lineage (for the 12 microsatellites that we tested them for) and not to a sympatric clonal lineage Comal Spring 8b.

Table S5.

	C101	VI/17	III/9	Weslaco	San Ignacio	Comal Spring 7a	Comal Spring 8b
C101	0.825	0.772	0.772	0.650	0.892	0.892	0.906
VI/17		0.992	0.989	0.750	0.791	0.791	0.750
III/9			0.997	0.708	0.788	0.788	0.747
Weslaco				1.000	0.667	0.667	0.625
San Ignacio					1.000	1.000	0.951
Comal Spring 7a						1.000	0.951
Comal Spring 8b							0.986

Table S5. Genetic Identity. The genetic identity (28) within each clonal lineage is higher (i.e., more closely related among each other; 1.000= 100% genetically identical within clonal lineage) than between clonal lineages, with exception of C101. The lack of higher genetic identity with the C101 clonal lineage may be reflected in the lack of behavioural evidence for kin recognition in this clonal lineage.

Table S6.

	C101	VI/17	III/9	Weslaco	San Ignacio	Comal Spring 7a	Comal Spring 8b
C101	0.115	-0.153	-0.153	-0.77	0.454	0.454	0.525
VI/17		0.960	0.944	-0.264	-0.057	-0.057	-0.264
III/9			0.985	-0.477	-0.072	-0.072	-0.279
Weslaco				1.000	-0.684	-0.684	-0.896
San Ignacio					1.000	1.000	0.752
Comal Spring 7a						1.000	0.752
Comal Spring 8b							0.929

Table S6. Relatedness Coefficient. The coefficient of relatedness (27) within each clonal lineage is higher than between clonal lineages, with exception of C101. R=1: identical twins/clones; R=0.5: clonal populations as related to each other as full siblings would be in an outcrossing, sexual species; and R<0: less identity than at random (i.e., individuals are as dissimilar to each other as unrelated individuals would be in outcrossing, sexual species with the lower numbers indicating the more unlikely related the lineages are). Note: the underlying logic of R is assuming sexual reproduction of diploid organisms, and therefore, these values are only considered an approximation in clonal organisms.